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ORIGINAL ARTICLES

ADVERSE REACTIONS TO INJECTED EMULSIFIED POLLEN EXTRACT IN ALLERGIC PATIENTS ON THE WEST COAST: RELATIONSHIP TO THE TOTAL DOSAGE OF ANTIGEN

Ben C. Eisenberg, M.D., Huntington Park, Calif., and*
*Walter R. MacLaren, M.D.,** Pasadena, Calif.*

FOR NEARLY a half century[1] allergic patients have been treated with good results for their inhalant sensitivities by means of repeated injections of specific aqueous extracts over a period of months or years. Since Spain's[2] efforts some 20 years ago to reduce the number of injections in the treatment of hay fever by using a slowly absorbed pollen extract in gelatin, others have tried to accomplish the same thing through the use of pollen tannates[3] and various suspensions and emulsions of antigens made with vegetable[4] or mineral oils.[5]

By incorporating large amounts of ragweed pollen extract in a modified form of Freund's adjuvant, Loveless[6] has been able to report success in the treatment of ragweed hay fever with a single repository injection each year. The dosage of ragweed pollen administered in this repository form was varied from 5,000 to 10,000 protein nitrogen units (PNU), according to the patient's sensitivity by test. In 1960, she reported[7] an incidence of 5 per cent adverse reactions when using repository injections, compared to 7 per cent following the conventional aqueous injections. The clinical effectiveness appeared to be the same with the use of either method of treatment.

Loveless[7] used three emulsion formulas containing emulsifier, mineral oil, and aqueous ragweed extract in the ratios by volume of 4:6:2, 1:6:5, and the

From the Allergy Clinic, Los Angeles County General Hospital, and the Medical Department, University of Southern California.

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*Associate Clinical Professor of Medicine, School of Medicine, University of Southern California.

**Associate Clinical Professor of Medicine, School of Medicine, University of Southern California, and Chief of Allergy Clinic, Los Angeles County General Hospital.

1:9:10 advocated by Brown.[12] The emulsifying agent was Arlacel A, the mineral oil was either Drakeol 6-VR or Atreol No. 9. The emulsions were prepared by forcing the mixture through a small orifice or by beating in a mechanical blender. Slight generalized reactions (considered desirable by Dr. Loveless) occurred in 24 of 128 patients treated with all types of the emulsion, an incidence of 18.7 per cent. The more serious reactions occurred mainly in the group of patients receiving the 1:9:10 formula. The formula 4:6:2 produced no such reactions.

No abscesses, cysts, or malignant degenerations were found by Loveless[6] to occur after 1,200 repository injections given during a 9-year period. The experience of Salk and others, as cited by Francis[8] has not shown mineral oil emulsions to be carcinogenic or to present any significant risk of producing sensitization. In addition, current investigation by the oil refining industry fails to show that injections of large amounts of highly refined mineral oil induces cancer in laboratory animals. Nevertheless, it must be kept in mind that long-term observation only will show whether or not there are late undesirable effects from injected mineral oil emulsions. Becker and his co-workers[9] have reported the appearance of a delayed type of sensitivity to ragweed pollen in normal, nonallergic human subjects (but not in clinically allergic individuals) after injections of the type of emulsion used to treat ragweed-sensitive patients.

Lowell has pointed out editorially[10] that unpredictable consequences, such as the appearance of polyarthritis and plasma cell tumors,[11] may follow the use in experimental animals of oily emulsion. How this applies to the use of emulsions in treating pollinosis in man is not clear, but it would be well to follow such patients closely for an extended period of time.

Our experience with repository treatment of allergy on the West Coast, and the incidence and causes of adverse reactions is the basis of this report.

Material and methods

The first emulsions we used contained Arlacel A,* Atreol No. 9,† and aqueous antigen extract in the ratios 4:6:2. They were prepared by repeatedly forcing the materials from a syringe through a 15 gauge needle pressed against the bottom of a test tube. Samples from a larger number of emulsions gave no bacterial growth when cultured in thioglycolate broth. Particle size, as checked microscopically, appeared to be satisfactory, and direct scratch tests with the emulsion on several highly sensitive patients showed little or no reaction.

Nevertheless the high rate of local and general reaction that accompanied the injection of these emulsions led us to change for a while to the formula 1:9:10, Arlacel A, Drakeol 6 VR,‡ and aqueous extract,[9] and to prepare the emulsion in a device that forces the material back and forth through "needles" of 18, 22, or 25 gauge (Brown Emulsor). During the final 6 months of this study we have used the formula 1:6:5—emulsifier: oil: extract—which has produced the most stable emulsion and the lowest incidence of reactions.

*Arlacel A (mannide mono-oleate, specially prepared) supplied through the courtesy of The Atlas Powder Co., Wilmington, Del.

†Atreol No. 9, Atlantic Refining Co., Newark, N. J.

‡Drakeol 6 VR, Pennsylvania Refining Co., Butler, Pa.

The choice of pollen antigens to be used in our standard mixtures was governed by the unusual position of Southern California as far as "antigenic vegetation" is concerned. Here grow more varieties of toxic pollen producing plants than anywhere else in the country. For example, there are more than twenty grasses that are active off and on during most of the year. Many victims of pollenosis begin their "spring season" in January, so that treatment may need to be started the preceding fall.

An analysis of the results of tests to pollens in several hundred patients showed the following to be the most commonly reacting plants:

Grasses:	*Agrostis alba* (bent)
	Cynodon dactylon (Bermuda)
	Festuca elatior (fescue)
	Lolium perenne (perennial rye)
	Poa annua (annual blue)
Weeds:	*Amaranthus retroflexus* (pigweed)
	Ambrosia psilostachya (Western ragweed)
	Artemesia vulgaris (California mugwort)
	Franseria acanthicarpa (burr ragweed)
	Salsola kali (Russian thistle)
Trees:	*Olea europea* (olive)
	Quercus agrifolia (live oak)
	Ulmus pumila (Chinese elm)

For treating grass allergy, emulsions were prepared which contained equal amounts of each of the grass pollens listed to make a total of 5,000 PNU or 10,000 PNU per cubic centimeter.

Weed pollen mixtures were prepared in the same strengths to match the sensitivities of the patients. In many cases, olive and elm pollen extracts were used individually, as these trees present special problems in this area.

The house dust and mold spore extracts came from various sources, and were assayed in terms of protein nitrogen units for the most part. Emulsions of these materials were prepared exactly as were those of pollen extracts.

In our experience, conjunctival reactivity to pollen antigen closely parallels that of the skin, so we used the results of scratch (puncture) and intradermal tests as the more convenient way of deciding how much antigen to give. Patients who showed negative scratch but positive intradermal tests were given at the beginning of the study 7,500 PNU to 10,000 PNU per injection; those strongly positive to scratch test were given 5,000 PNU. These limits were chosen because ragweed-sensitive patients in the East appeared to tolerate reasonably well this range of dosage. The doses were increased or decreased as the study progressed, depending on the number and severity of the reactions encountered.

The amount of emulsion injected was initially set at 0.5 to 1.0 c.c. Later amounts as little as 0.2 c.c. were used, but these were given up because of the increased number of reactions that follow the injection of a large amount of antigen in a small amount of emulsion. For the major part of the study the original plan was followed.

RESULTS

Adverse or over-dose reactions were either local or generalized. Local reactions varied in intensity from a barely noticeable swelling and itching to erythema and painful swelling involving most of the upper arm, with blister formation directly over the emulsion mass. The mild local reactions were ignored, but painful swelling and erythema involving half the arm was considered to indicate over-dose. The large local reactions were fairly common occurrences with the earlier emulsions we used. Signs and symptoms usually persisted for 2 to 14 days, although at least one in twenty lasted for a month. The more severe long lasting local reactions occurred only in patients showing marked sensitivity by scratch test. However, no abscesses, cysts, draining sinuses, or malignancies have appeared.

Generalized reactions presented the same picture as is found to follow an over-dose of aqueous pollen extract—various combinations of such symptoms and signs as coryza, flushing, generalized itching and urticaria, angioedema, headache, chest pain, wheezing, dyspnea, intestinal cramping, nausea, vomiting, diarrhea, and hypotensive shock. This type of reaction usually began within 2 hours after administration of the injection, although we have had instances starting within 15 minutes, or delayed as much as 8 hours.

Even severe reactions usually subsided in 10 to 20 minutes after an epinephrine injection or two. When symptoms continued or recurred, oral antihistamines, sublingual isopropylarterenol, and, in a few cases, adrenal corticosteroids were given for 1 or 2 days. Only one patient, a woman of 40 years of age, with asthma of long standing was sent to the hospital during a reaction when it seemed she might need an emergency tracheotomy in the face of increasing edema of the tongue and larynx. (Fortunately the edema subsided rapidly under treatment with epinephrine and antihistamines, and for the first time in her life the patient was afforded a period of relief from asthma.)

Six of the first 30 patients (20 per cent) treated for pollen allergy suffered from moderately severe generalized reactions, so the dosage used was progressively lowered. Doses of 2,500 to 5,000 PNU caused general reactions in about 10 per cent of cases in the early part of the study; doses of 1,250 PNU in 6 per cent. When 1,000 PNU or less was given, no reactions occurred. Accordingly in order to avoid serious reactions all patients strongly positive by test to pollens were given no more than 1,000 PNU at a time.

Even greater care appeared necessary when treating patients sensitive to Chinese elm (*Ulmus pumila*) pollen. These trees, which pollinate in August and September, have become very popular in the last few years and are plentiful along parkways and close to houses in residential areas. They produce large amounts of a very antigenic pollen which is responsible for severe symptoms. Many patients are sensitive to this pollen alone. Doses of emulsified elm pollen extract between 1,250 PNU and 2,500 PNU produced serious constitutional reactions in 6 of 24 patients, an incidence of 25 per cent. It was necessary to keep the amount injected below 1,000 PNU in order to avoid reactions.

It is worth noting that, with the adoption of the formula 1:6:5—Arlacel A: Drakeol 6 VR: aqueous extract—and the use of the Brown Emulsor to prepare the emulsions, the rate of reactions following injections significantly declined, so that in the second half year of the study it was possible to increase safely the amount of antigen given. Moderately sensitive patients were able to tolerate doses of 5,000 PNU or more of pollen extract; however, those very sensitive were limited to 2,500 PNU at a time if severe reactions were to be consistently avoided. During the period from October, 1960, through January, 1961, only 10 generalized reactions followed 435 repository injections.

This data is summarized in Table I which sets forth the number of adverse reactions encountered according to the dosage of various antigens. The over-all incidence of reactions, from November, 1959, to January, 1961, was 4.69 per cent.

TABLE I. SUMMARY OF 1,279 EMULSIFIED POLLEN, HOUSE DUST AND MOLD EXTRACT REPOSITORY TREATMENTS ADMINISTERED TO 850 ALLERGIC PATIENTS FROM NOV. 10, 1959, TO JAN. 12, 1961

PNU	GRASS		TREES		WEEDS		HOUSE DUST		MOLDS	
RANGE OF DOSAGE	NO. OF INJECTIONS	ADVERSE REACTIONS	NO. OF INJECTIONS	ADVERSE REACTIONS	NO. OF INJECTIONS	ADVERSE REACTIONS	NO. OF INJECTIONS	ADVERSE REACTIONS	NO. OF INJECTIONS	ADVERSE REACTIONS
250-1,250	380	15	34	0	66	0	22	0	15	1
1,300-2,500	14	2	39	6	273	2	18	1	31	3
3,000-7,500	49	4	12	2	22	2	146	4		
Totals	578	29 (5%)	97	10 (10%)	364	7 (2%)	194	10 (5%)	46	4 (9%)
Total No. Repository Injections							1,279			
Total No. Adverse Reactions							60 (4.69%)			

DISCUSSION

Examination of our experience and that reported by others suggests that the main causes of adverse reactions after emulsified antigen injections are these:

1. Poor quality emulsions: inadequate mixing, wrong proportions, impurities, breakdown, and aging of once good emulsions.
2. Excessive dose of antigen: good emulsion, but amount of aqueous phase inevitably absorbed from the surface of the emulsion exceeds patient's tolerance.
3. Improper timing of treatment: injection given at the time of heavy natural exposure to the antigen, namely, the height of the pollen season.
4. Incorrect technique of injection; emulsion deposited intracutaneously or intramuscularly.
5. Patient unusually "fragile" due to multiple or extreme sensitivity, or under unusual exposure to antigen, or consuming alcohol, or suffering from concomitant serious disease.

As the quality of emulsion improves with the discovery of more stable ingredients the first cause will doubtless become of minor importance. Decisions as to the correct dose of antigen will, as always, have to be based on the

experience and skill of the physician. The proper timing of the injections is complicated in an area where many antigens are constantly coming and going, and calls for a good knowledge of the prevailing flora and of the individual's exposure to allergens.

The importance of concomitant disease is well illustrated by the following case. A woman who had suffered through several episodes of multiple sclerosis was given 2,500 PNU of emulsified grass pollen allergen for her allergic rhinitis. Within a few minutes after the injection, she complained of "burning" at the site, began violent sneezing, broke out with large welts everywhere, was unable to breathe through her nose and felt extreme pressure in her head and ears, noted numbness and pain in the extremities, and showed signs of moderate peripheral vascular collapse. She recovered after 2 hours, during which time she was given oxygen, Adrenalin injections, antihistamines, metaraminol, and dexamethasone.

Very soon after starting our work with repository treatments it became evident that we could not, even with the same type and quality emulsions, administer the high order of dosage as given in the East and Mid-western United States. While previous investigators have usually given their patients 5,000 to 10,000 PNU of emulsified pollen extract with few reported incidents of constitutional reactions, we have encountered as high as 25 per cent adverse effects, some serious, when attempting to give these amounts. With the advent of improved emulsifying techniques the incidence of untoward reactions has decreased and we have been able to give higher doses of pollen extract. Using Brown's Emulsor and Loveless' new formula (1:6:5), we have prepared emulsions that allow us to give to the average patient 5,000 PNU or either grass, tree, or weed pollen antigen. The very sensitive individual may now receive up to 2,500 PNU while the least sensitive patient, who is judged not too "fragile" or allergic to too many substances, may get as much as 7,500 PNU.

It must be emphasized, however, that regardless of how good the emulsion may be one must treat the highly allergic person, and those with serious concomitant disease (for example, multiple sclerosis) with extreme caution, particularly at or near the height of a bad season, or while symptoms have not yet been brought under control. We should learn to have the utmost respect for those patients who show markedly positive scratch or intradermal tests. Especially must we be wary of the patient who reports anything resembling a constitutional reaction, no matter how mild, following the skin testing procedures during a routine allergy work-up. This exquisite sensitivity may predispose this type of patient to a great hazard should the antigen-containing aqueous (internal) phase of the water in oil emulsion lose enough of its protective oily envelope during the process of administration or after contact with the patient's tissues. Complex physicochemical forces, such as tissue enzymes, cannot be excluded as possible influences on the rapid release of the aqueous phase.

Every allergist who attempts to treat with emulsified extracts should acquaint himself with the fundamentals of emulsion chemistry, understand the nature and action of the various emulsifying agents, and learn how to determine particle size through use of the microscope. In not too many years, repository

treatment may well replace present conventional methods of immunization, including vaccine therapy as well as specific allergy treatment. There is much more involved in the repository method of hyposensitization than merely reaching for a disposable cartridge and injecting a ready-made oil emulsion containing dangerous, active material. We must know our patient, his history, general condition, degree of sensitivity, associated conditions and sensitivities, and even his emotional make-up.

Concerning benefit from repository treatments given our patients sensitive to grass, tree, and weed pollen, it is not possible from one year's observation alone to evaluate the results in the perennial allergic sufferer with multiple sensitivities. He should, if possible, receive vigorous anti-allergic therapy directed against other factors of etiologic significance (such as house dust and molds) before one can properly assess the value of treatment using emulsified pollen extracts. Double-blind studies presently underway at the Los Angeles County General Hospital will provide us with the answer to this question. The main purpose for this past year's investigation has been the establishment of a safe maximum dosage schedule of emulsified pollen extract with which to treat our allergy patients, recognition and treatment of over-dose reactions, and to learn to prepare the best possible emulsion for use in human beings.

SUMMARY

A group of 850 patients suffering from allergy to one or more of the three major classes of pollens (namely, grasses, trees, or weeds), house dust, or mold spores were treated by means of injections three or four times a year of emulsified extract of the specific antigens to which they showed sensitivity. Grass-sensitive patients received their repository injections from November to September; patients sensitive to tree pollen from January to April; sufferers from allergy to weed pollen from April to August. Individuals allergic to Chinese elm, which pollinates in August and September, were given emulsified elm pollen extract in July and August.

Manually prepared emulsions, used in the beginning, produced over-dose reactions in 20 per cent of patients receiving 5,000 or more protein nitrogen units of pollen extract; in 10 per cent of those given 2,500 to 5,000 PNU; and in 6 per cent of those given 1,250 PNU; injections of 1,000 PNU or less caused no reactions.

Out of a total of 1,279 repository treatments given from November, 1959, to January, 1961, there has been an over-all adverse reaction rate of 4.69 per cent. After the administration of 435 repository treatments of mechanically prepared emulsions given since Oct. 1, 1960, however, the incidence of adverse reaction has been only 2.3 per cent.

Those patients with the most markedly positive skin tests, especially to scratch, showed the more serious types of adverse reactions. Also, individuals suffering from concomitant disease should be treated cautiously.

The effectiveness of repository injections in the treatment of multi-allergic patients must await further study before it can be properly assessed.

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DESENSITIZATION WITH HAPTENS

Role of Molecular Size in Specific Precipitation, Anaphylaxis, and Desensitization With Dextran Fractions

Rudolf Hoene, M.D., Ph.D., Oscar Swineford, Jr., M.D.,** and Susan Quelch, B.S., Charlottesville, Va.*

SENSITIZED ANIMALS have been made tolerant of multiples of normally fatal doses of allergens in a variety of ways, all of which were transient. Theoretically, lasting desensitization should follow neutralization of sensitizing antibody with specific haptens. This is one of a series of studies of desensitization with haptens,[1-5] the goal of which is to produce permanent desensitization in actively sensitized animals.

Dextran is an allergen in man.[6] This limits its usefulness as a plasma expander. It seemed appropriate to study the problem of desensitization of dextran-sensitive animals with a series of haptenic fractions of dextran. Dextran seemed particularly suited to this type of experiment because it has been shown that: (1) it can elicit anaphylaxis in and desensitize passively sensitized guinea pigs specifically[5]; (2) partial hydrolysis results in striking diminution of the capacity of native dextrans to produce wheal and flare reactions in man[6]; (3) dextran fractions with average molecular weights of 3,000 to 4,000 precipitated much less rabbit anti-*Leuconostoc mesenteroides* antibody than did larger dextrans[7]; (4) dextran fractions with average molecular weights of about 10,000 removed only about two fifths as much precipitable antibody as did the native dextran[8a]; (5) very small dextran fractions (isomaltodextrins) will inhibit precipitation in the human anti-dextran system[8b]; and (6) low molecular haptens could produce transient desensitization.[9]

Those observations suggested the possibility that dextran fractions would desensitize passively sensitized guinea pigs and that, if this were so, their desensitizing capacities might be related to their molecular weights and to their capacities to precipitate, to inhibit precipitation, and to elicit anaphylaxis. Accordingly native dextran was subjected to a series of fractionation procedures.

From the University of Virginia School of Medicine, Charlottesville, Va.

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*Former Fellow in Allergy-Arthritis, Department of Internal Medicine. Present address: The Golden Clinic, Elkins, West Va.

**From the Allergy Research Laboratory, Department of Internal Medicine, University of Virginia School of Medicine, Charlottesville, Va.

Studies of these fractions showed that progressive molecular cleavage transformed dextran from: (1) an anaphylactogen to anaphylactogenically inactive fractions, (2) an anaphylactogen to specific inhibitors of anaphylaxis, and (3) a specific precipitator to specific inhibitors of precipitation.

MATERIALS AND METHODS

*Antiserum.**—A pneumococcus Type II rabbit serum, which cross precipitated with dextran,[10, 11] was used (1) to sensitize the guinea pigs used to study the anaphylaxis-provoking and desensitizing effects of low molecular weight dextran fractions; (2) to study specific precipitation by native dextran and its low molecular weight fractions, and (3) to study the ability of low molecular weight dextran to inhibit immune precipitation with native dextran.

Dextran.—Native enzymatically synthesized NRRL B 512-E-SR dextran and clinical dextran, prepared from the same B-512 strain of *L. mesenteroides* by Wyeth Laboratories, were used for the preparation of low molecular dextrans by hydrolytic (acid and enzymatic) degradation and subsequent fractional precipitation. The variations in size of the low molecular fractions were narrowed by precipitation with alcohol and ether, eliminating "head and tail" fractions. Since the individual dextran molecule has only one reducing group, the *Number Average Molecular Weight* (Mn) was determined, as outlined by Isbell and co-workers,[12] using the Folin-Wu method and anhydrous dextrose as reference standard. Technical data on the dextran fractions are summarized in Table I.

Quantitative Immune Precipitation.—Measured quantities of dextran fractions (abscissa, Fig. 1) were dissolved in 1.0 ml. saline, added to 0.5 ml. diluted antiserum (1:3 with saline), and incubated for one hour at 37° C. and then for 24 hours at 0° C. The precipitates were then collected by centrifugation, washed once with chilled saline, and dissolved in 0.1 normal NaOH. The density of the solution was read (Beckman, Model DU with photomultiplier tube, 288 mμ wave length, 0.5 mm. slit width). The values obtained were converted to immune precipitate nitrogen (ordinate, Fig. 1) by means of the extinction coefficient for rabbit antibody $E\ \frac{1\%}{1\ cm.}$, which was found to be 16.4 under our conditions.

Inhibition Assays.—Measured quantities of the low molecular dextran fractions (dissolved in 0.5 ml. saline) were added to 0.5 ml. of diluted antiserum (see Fig. 2). After 10 minutes at 37° C., a quantity of native dextran (SR) in 0.5 ml. saline was added to each tube, and also to tubes containing the same amount of antiserum but no low molecular dextran. After further incubation (1 hr. at 37° C., 24 hr. at 0° C.) the immune precipitate was collected and measured as described in the previous paragraph.

*The generous supplies of this serum (Wyeth No. 461) and clinical dextran donated by Wyeth Laboratories in Marietta, Pa., are gratefully acknowledged. The serum was found to contain 3,150 mcg. Ab N per milliliter precipitating with the homologous polysaccharide.

Assay of the Challenging and Desensitizing Effects in Anaphylaxis.—Forty-six guinea pigs, weighing between 180 and 230 grams, were passively sensitized by intraperitoneal injections of 1.0 ml. of diluted antipneumococcal Type II serum (Wyeth No. 461) containing 200 meg. of anti-native dextran-precipitating antibody nitrogen. This also contained 70 to 80 meg. of anti-fraction DF_1 precipitating antibody nitrogen, but no precipitating antibody against fractions HF_1 and HF_5. Forty-eight hours later, known quantities of native dextran and low molecular dextran fractions dissolved in 0.1 ml. saline (see Table II) were injected into a saphenous vein, keeping the injection time as close to 8 to 10 seconds as possible. The challenging effects of native and low molecular dextran fractions were compared. The low molecular fractions were injected in large quantities for better demonstration of any anaphylactogenic action. All animals which had received the low molecular weight fractions were re-injected with native dextran at specified time intervals (see Table III) for evaluation of any desensitizing effects of the low molecular weight dextrans. The degree and duration of desensitization were observed.

TABLE I. TECHNICAL DATA ON PREPARATION AND MOLECULAR WEIGHTS OF THE DEXTRAN FRACTIONS

FRACTION	HYDROLYSIS	ALCOHOL FRACTIONATION	Mn*
B-512-E SR native†	0	insol. 36.6 - 38.5% ethanol	9 Mill.‡
B-512-F DF_1	10 per cent solution of clinical dextran (Wyeth) in 0.1 N HCl, 99° C. for 60 min. under reflux	insol. 30 - 40% isopropanol	14,000
B-512-F DF_2		insol. 40 - 50% i.p.a.	8,500
B-512-F DF_3		insol. 50 - 60% i.p.a.	5,000
B-512-F DF_4		insol. 60 - 80% i.p.a.	2,200
B-512-F HF_1	10 per cent solution of clinical dextran (Wyeth) in 0.4 N HCl, 99° C. for 135 min. under reflux	insol. 50 - 60% i.p.a.	1,500
B-512-F HF_3		insol. 70 - 80% i.p.a.	950
B-512-F HF_5		insol. 90 - 95% ether (of total vol.)	850
B-512-F EH_1	24 hr. at 25° C.	10 per cent solution of clinical dextran (Wyeth) in buffered saline pH5; 1 mg. dextranase (*Penicillium funiculosum* NRRL 1,768§) per 20 Gm. dextran	insol. 30 - 40% i.p.a.	17,000
B-512-F EH_2	48 hr. at 25° C.		insol. 40 - 50% i.p.a.	8,700
B-512-F EH_3			insol. 50 - 60% i.p.a.	5,400
B-512-F EH_4			insol. 60 - 80% i.p.a.	2,900

*Number average molecular weight.

†Enzymatically synthetized. Made available by Dr. C. A. Wilham, U.S. Agricultural Research Service, Peoria, Ill.

‡Based on light scattering determination.

§Made available by Dr. H. M. Tsuchiya, U.S. Agricultural Research Service, Peoria, Ill.

RESULTS

Technical details of hydrolysis, isolation, and the molecular weights of the individual dextran fractions are given in Table I. It can be seen that the fractions obtained by mild acid and heat hydrolysis (DF_1 to DF_4) and the fractions obtained by enzymatic hydrolysis (EH_1 to EH_4) have approximately the same molecular size. Intensified acid and heat hydrolysis and subsequent isopropyl alcohol fractionation seemed to result in fractions of somewhat lower molecular size (HF_1 to HF_5). Fraction HF_1 with an average molecular weight of 1,500 may be assumed to consist chiefly of isomaltooctaose and an occasional larger polymer. Fractions HF_3 and HF_5 with average molecular weights of 950 and 850, respectively, probably contained isomaltodextrins ranging from isomaltotriose to pentaose, with an occasional larger polymer. Although the number average molecular weight (Mn) does not give the exact degree of polydispersity, it was believed that estimates of the molecular sizes in the individual dextran fractions, based on the technical data in Table I, would be sufficiently accurate for these experiments.

Precipitating Effects.—The precipitation curves for fractions SR, DF_1 – DF_4, and EF_1 – EF_4, reacting with the rabbit antipneumococcal Type II serum are given in Fig. 1. Whereas the large molecular dextran SR (molecular weight 9,900,000) precipitated 98 meg. antibody nitrogen at the point of maximum precipitation, fraction DF_1 (Mn 14,000) precipitated only 35 meg.; fraction DF_2 (Mn 8,500) precipitated 19 meg.; fraction DF_3 (Mn 5,000) precipitated 5 meg. antibody nitrogen; fraction DF_4 (Mn 2,200) caused no precipitation. The enzymatically obtained fractions EH_1 – EH_4 showed a similar progressive loss of immune precipitating effect with decreasing molecular weight. This effect seemed slightly better preserved in the EH fractions than in the comparable DF fractions. This may have been due to the fact that the molecular size of the EH fractions was slightly larger than in the corresponding DF fractions. It will be noted also in Fig. 1 that the point of maximum precipitation is reached in the small molecular fractions at lower antigen concentrations than in the larger fractions. This is consistent with the idea that the precipitation was due primarily to the smaller molecules, but does not rule out a significant contribution by larger molecules. It is also consistent with the development of inhibition by small fragments of dextrans. None of the HF fractions had any precipitating effect in this immune system, so they were not included in Fig. 1.

Specific Inhibition of Immune Precipitation.—The small molecular weight dextran fractions were studied for their capacities to inhibit specific precipitation in this pneumococcus Type II antiserum-native (SR) dextran system (Fig. 2). There was no detectable difference in the inhibitory effect of dextran fractions DF_1 to HF_3, with molecular weights ranging from 14,000 to 950. Therefore, the inhibition of immune precipitation by fraction HF_3, in the upper part of Fig. 2, exemplifies also the inhibition by the low molecular fractions DF_1 to HF_1 (Table I). Curve SR shows the immune precipitation of the native

dextran. The lower curves in the upper half of Fig. 2 show the amount of precipitation caused by adding 500 or 2,000 meg. HF_3 to the antiserum before SR was added. Note that 2,000 meg. of the HF_3 low molecular (Mn 950) dextran inhibited immune precipitation completely. This was roughly twenty times the amount of native dextran which gave maximum precipitation. The

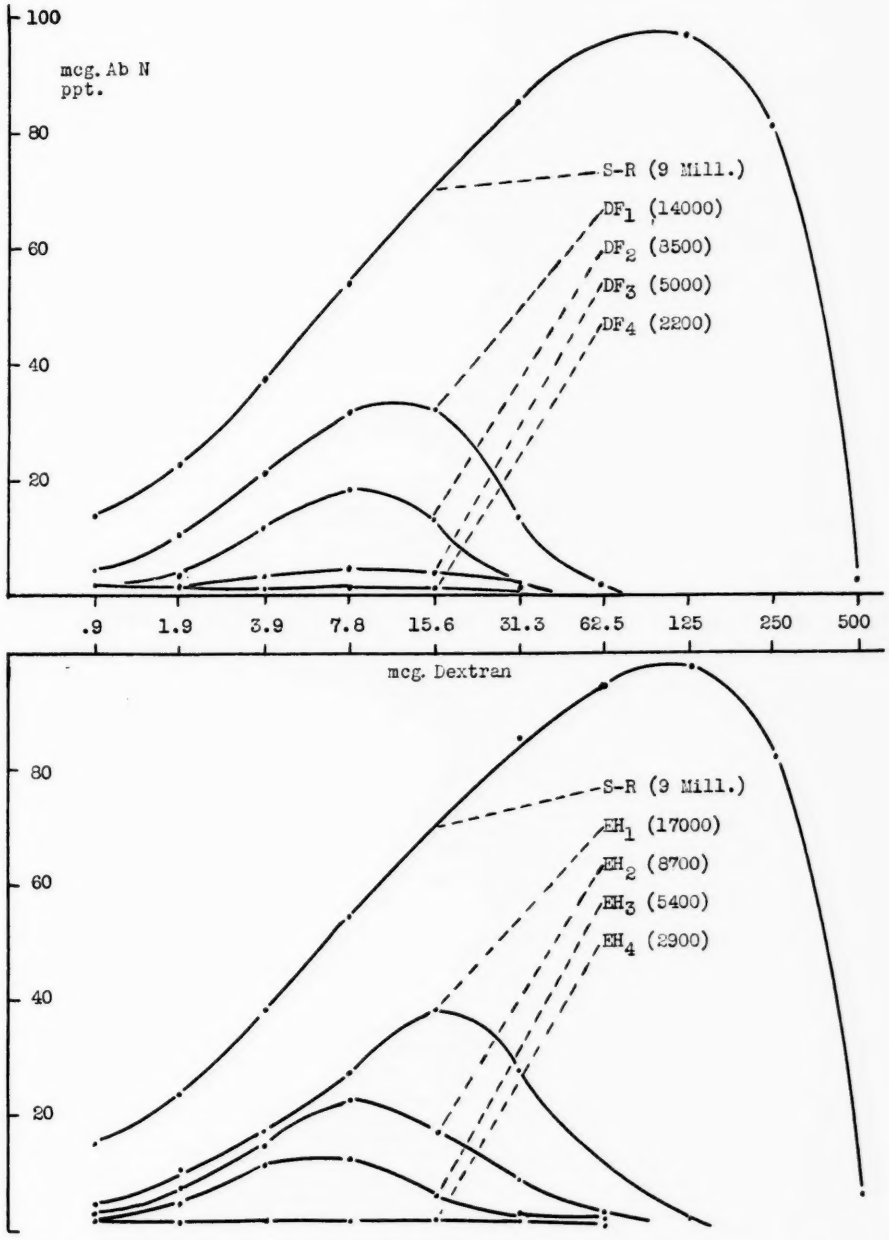

Fig. 1.—Immune precipitation of dextran fractions, effect of decreasing molecular weight (see text).

lower half of the figure demonstrates that fraction HF_5 had much less inhibitory activity than fraction HF_3. But the addition of even 8,000 mcg. of HF_5 to the antiserum did not abolish precipitation entirely.

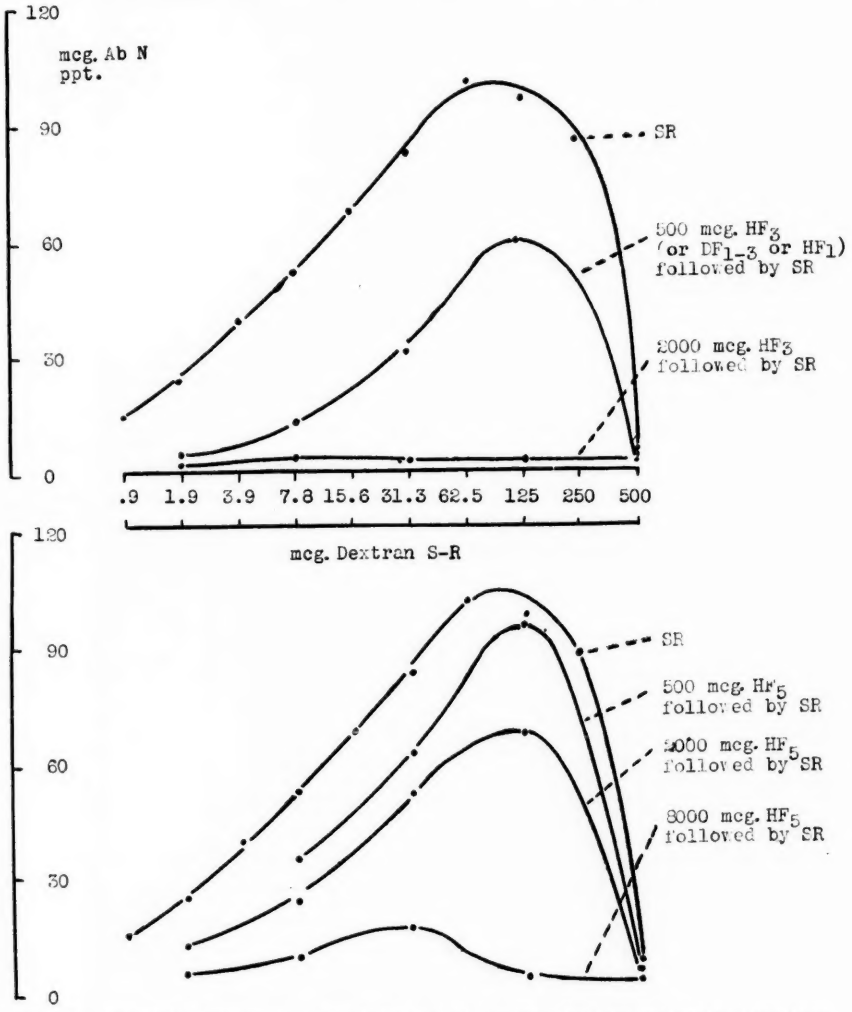

Fig. 2.—Inhibition of immune precipitation of dextran by low molecular fractions.

Challenging and Desensitizing Effects of the Fractions in Passive Anaphylaxis.—Guinea pigs passively sensitized by intraperitoneal injections of rabbit antipneumococcal Type II serum were challenged by intravenous injections of dextran fractions 48 or 72 hours later. As seen in Table II, 1.66 mg. of native SR dextran produced fatal anaphylaxis in 3 sensitized guinea pigs; 0.55 mg. killed 11 of 12 animals, while one exhibited a severe anaphylactic shock. When challenged with 0.18 mg. of native dextran, one animal died, one had a moderate reaction, and 2 exhibited no anaphylactic symptoms. It was concluded that a challenging dose of 0.55 mg. native dextran could be expected to elicit fatal anaphylaxis in the large majority of animals and could be considered to be approximately LD_{90}.

TABLE II. ANAPHYLACTOGENIC EFFECTS OF DEXTRAN FRACTIONS*

NO. OF GUINEA PIGS	INTERVAL AFTER SENSITIZATION (HR.)	FRACTION USED (MOL. WT.)	CHALLENGE DOSE I.V. (MG.)	ANAPHYLACTIC REACTION			
				AD†	SEVERE	MODERATE	NONE
3	48	Native SR (9 Mill.)	1.66	3			
8	48		0.55	7	1		
4	72		0.55	4			
4	48		0.18	1		1	2
10	48	DF_1 (14,000)	50.0			1	9
8	48	HF_1 (1,500)	50.0				8
9	48	HF_5 (850)	50.0				9

*Passive sensitization: 1.0 ml. diluted rabbit antipneumococcal Type II serum intraperitoneally, containing 200 mcg. ab N precipitatiing with native SR dextran or 70-80 mcg. Ab N precipitating with fraction DF_1.

†Anaphylactic death.

The effects of Wyeth clinical dextran in anaphylaxis and precipitation (not shown in Fig. 1 and Table II) were found to approximate those of native dextran very closely.

Neither of the low molecular fractions (HF_1 and HF_5) produced anaphylactic reactions in the passively sensitized guinea pigs even when 100 times the LD_{90} of native dextran (Table II) was given. Fraction DF_1 elicited a moderate anaphylactic reaction in 1 of 10 animals.

The desensitizing effect of the low molecular dextrans was then tested by challenging, with native SR dextran, those guinea pigs which had been given the large doses of fractions DF_1, HF_1, HF_5 (Table III). The time interval between the injection of the low molecular fractions and the native SR dextran varied between 14 min. and 24 hr. The challenging doses of native SR dextran were 1 or 3 LD_{90} (0.55 mg. and 1.66 mg.). It may be seen that native dextran elicited no anaphylactic reaction in the group which had been given the DF_1 (Mn 14,000) fraction previously. Apparently the large dose of the low molecular DF_1 fraction had desensitized them for at least 24 hours. In the

TABLE III. INHIBITION OF ANAPHYLAXIS BY LOW MOLECULAR DEXTRAN FRACTIONS (DESENSITIZATION)*

GUINEA NO. OF PIGS	LOW MOLECULAR FRACTION (DOSE)	RE-CHALLENGE		ANAPHYLACTIC REACTION				DESENSITIZATION
		TIME AFTER 1ST CHALLENGE	DOSE OF SR (MG.)	AD†	SEVERE	MODERATE	NONE	
3	DF_1 50 mg.	20-30 min.	0.55				3	Effective and
3	DF_1 50 mg.	20-30 min.	1.66				3	lasting
4	DF_1 50 mg.	24 hr.	1.66				4	> 24 hr.
2	HF_1 50 mg.	15-20 min.	0.55				2	Effective but
3	HF_1 50 mg.	15-20 min.	1.66				3	transient;
2	HF_1 50 mg.	3 hr.	1.66	2				> 20 min.,
1	HF_1 50 mg.	3 hr.	0.55	1				< 3 hr.
1	HF_5 50 mg.	14 min.	0.55				1	Weak and
3	HF_5 50 mg.	10-20 min.	1.66		3			transient;
5	HF_5 50 mg.	20-40 min.	1.66	4		1		> 14 min., < 40 min.

*The passively sensitized guinea pigs, after injection with the low molecular dextrans, received 1 or 3 LD_{90} of native SR dextran.

†Anaphylactic death.

group given fraction HF_1 (Mn 1,500) the desensitization was transient. All of those animals died when rechallenged with the native dextran 3 hours after they had received the HF_1 dextran. The desensitization, if any, afforded by the HF_5 (Mn 850) fraction was weak and was almost gone when the pigs were rechallenged 20 to 40 min. later.

DISCUSSION

The decrease in precipitating activity with decreasing molecular size of B-512 dextran fractions is illustrated in Fig. 1. No significant difference was observed when the precipitation curves obtained with low molecular weight dextrans produced by acid and heat hydrolysis were compared with the curves of the enzymatically hydrolyzed dextrans of corresponding molecular size. Fractions DF_1 and EH_1, with average molecular weights of 14,000 and 17,000, respectively, precipitated less than 50 per cent as much antibody (35 meg.) as was precipitated by native SR dextran (98 meg.). Some precipitating activity remained in fractions DF_3 and EH_3 with average molecular weights of 5,000 to 5,400. But at the average molecular weights of 2,200 to 2,900, the fractions ceased to produce precipitates. These observations are similar to those of Glynn and associates[7] that dextran of an average molecular weight of 3,000 to 4,000 was markedly less active in precipitating rabbit dextran antibody than was dextran of an average molecular weight of 25,000. Our results are also in agreement with those obtained by Goodman and Kabat.[8a] Their fraction 2 (average molecular weight 35,000) precipitated 42 per cent of the antipneumococcal antibody nitrogen precipitable by native dextran B-512. Their fraction 1 (average molecular weight 10,000) precipitated 41 per cent of the antibody nitrogen precipitable by native B-512 dextran. Our DF_1 fraction (Mn 14,000) precipitated 36 per cent of the pneumococcal antibody nitrogen precipitable by native B-512 dextran. The small difference may be accounted for, in part, by our shorter incubation and aging period (24 hours at 0° C. instead of their 7 days) prior to the collection of the immune precipitate.

The effect of our low molecular weight dextran fractions in inhibiting immune precipitation of native SR dextran was about the same for the range of 14,000 to 950 Mn. The marked decrease of the inhibitory effect of HF_5 (Mn 850) suggests that this fraction consisted of isomaltodextrins, the majority of which were probably smaller than isomalto-pentaose, which was shown by Goodman and Kabat[8c] to be the most effective inhibitor in this cross-precipitating system.

One purpose of this investigation was to determine if there was any correlation between the progressively reduced molecular weights of the dextran fractions and the loss of their precipitating, anaphylactogenic, and desensitizing powers. This was planned in the hope that such a correlation would lead to easy and reliable predictions of the desensitizing capacity of a variety of haptens.

The interesting observation was made that, with decreasing molecular size, the anaphylactogenic effect of the dextran fractions was apparently lost sooner

than the precipitating effect. This is shown by the fact that fraction DF_1 (Mn 14,000) produced (nonfatal) anaphylaxis in only one of 10 passively sensitized guinea pigs, although it precipitated 36 per cent as much antibody as was precipitated by the native dextran. Those pigs were sensitized with 200 meg. of precipitating anti-native dextran antibody nitrogen, or about 75 meg. of antibody nitrogen precipitable by fraction DF_1. It had been shown previously that 55 meg. anti-dextran antibody nitrogen from this same Type II antiserum would sensitize guinea pigs regularly against native dextran.[5] Fatal anaphylactic sensitization has been induced in guinea pigs by Kabat and Benacerraf[13] with as little as 30 meg. of antibody nitrogen. It is difficult to explain the failure of 75 meg. of precipitating anti-DF_1 antibody nitrogen (plus an unmeasured amount of univalent anti-DF_1 antibody) to sensitize pigs effectively against large amounts of DF_1 (Mn 14,000), even when allowances are made for the differences in sensitizing capacity of various antisera. Ovary and Karush[14] reported a somewhat similar inability to provoke passive cutaneous anaphylaxis with monovalent haptens (not dextrans).

There was no apparent correlation between the capacities of the low molecular dextran fractions to inhibit immune precipitation and to desensitize guinea pigs. For example, fraction HF_1 (Mn 1,500) inhibited immune precipitation as well as did fraction DF_1 with a molecular weight of 14,000. But DF_1 (Mn 14,000) maintained desensitization much more effectively than fraction HF_1 (Mn 1,500). By the same token, large amounts of fraction HF_5 (Mn 850) still inhibited immune precipitation, whereas the desensitizing effect of HF_5 was negligible. The short duration of specific desensitization with low molecular haptens is in accord with the early observations of Tillet, Avery, and Goebel.[9] Ovary and Karush,[14] who studied the role of other haptens in passive cutaneous anaphylaxis, thought that "the transitory protection by the hapten is due to its ready dissociation from antibody (and its rapid removal from the site of reaction) . . . which is thus rendered available for further reaction with the complete antigen."

These experiments indicate that lasting desensitization with related polysaccharides should be sought in haptens with molecular weights in excess of 14,000.

The Mn 14,000 dextran fraction was weakly anaphylactogenic, but the Mn 1,500 fraction was not. Both of these fractions produced desensitization. Dextran is an allergen in man. This limits its usefulness as a plasma expander. These findings suggest that reactions to infusions of clinical dextran could be prevented by prior desensitizing infusions of dextran fractions, with molecular weights larger than 1,500 and smaller than 14,000.

SUMMARY

Clinical dextran was subjected to varying degrees of acid and enzymatic hydrolysis and fractionation with alcohol and ether. The number average molecular weights (Mn) of the different fractions varied from 17,000 to 850.

Cross-precipitation with Type II antipneumococcal rabbit serum decreased with the molecular size of the fractions. Precipitation occurred with the Mn 5,000 but not with the Mn 2,200 fraction.

Precipitation was inhibited about equally by fractions Mn 14,000 to 950. There was much less inhibition by a smaller fraction (Mn 850.)

Native and clinical dextrans elicited fatal anaphylactic shock regularly in guinea pigs which had been passively sensitized with Type II antipneumococcal rabbit serum. The Mn 14,000 dextran fraction elicited a moderate reaction in only one of 10 similarly sensitized animals. Smaller fractions, even in large doses, did not provoke anaphylaxis at all. Varying degrees of desensitization against native dextran followed large challenging doses of several of the fractions. Desensitization was complete for 24 hours with the Mn 14,000 fraction, complete but transient (less than 3 hours) with the Mn 1,500, and negligible with the Mn 850 fraction.

Studies of these fractions showed that progressive molecular cleavage transformed dextran from (1) an anaphylactogen to anaphylactogenically inactive fractions, (2) an anaphylactogen to specific inhibitors of anaphylaxis, and (3) a specific precipitator to specific inhibitors of precipitation. But there was no parallelism between the progressive reduction in molecular weights of the fractions and the loss of precipitating, anaphylactogenic inhibitory and desensitizing powers.

Dextran is an allergen in man. This limits its usefulness as a plasma expander. These findings suggest the need to investigate the possibility that reactions to infusions of clinical dextran could be prevented by prior desensitizing infusions of dextran fractions, with molecular weights larger than 1,500 and smaller than 14,000.

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THE INCIDENCE OF POSITIVE INTRADERMAL REACTIONS AND THE DEMONSTRATION OF SKIN SENSITIZING ANTIBODY TO EXTRACTS OF RAGWEED AND DUST IN HUMANS WITHOUT HISTORY OF RHINITIS OR ASTHMA

John H. Lindblad and Richard S. Farr, M.D.,** Pittsburgh, Pa.*

DESPITE the importance of the intradermal test as a diagnostic aid for the identification of causative agents in allergic rhinitis and asthma, only a few studies are available in which the incidence of positive reactions has been recorded when the extracts used for such testing are studied on individuals without rhinitis or asthma. Some of these important control studies were concerned with only one concentration of multiple allergens,[1, 2, 3] a few with multiple concentrations of a single allergen,[4, 5] but none have been concerned with multiple concentrations of several allergens tested on the same group of individuals. Accordingly, the present experiment was undertaken to determine the incidence of positive intradermal tests when graded concentrations of extracts from ragweed, mixed grasses, mixed trees, mixed molds, and dust were injected into the skin of individuals who denied ever suffering from the usual nasal or pulmonary symptoms thought to be associated with allergic phenomena. Some additional studies were also done to determine whether some of the positive reactions observed were due to nonspecific irritation, or were due to the presence of passively transferable skin sensitizing antibody.

METHOD

Test Subjects.—Subjects for this investigation were chosen at random from graduate nurses, medical students, patients attending Falk Medical Out-Patient Clinic, and patients admitted to the ward of Presbyterian Hospital, Pittsburgh, Pa. The criteria used for including a given subject in this study were (a) a completely negative history of nasal or respiratory symptoms which could in any way be interpreted as being on an allergic basis, (b) the absence of steroid, bronchodilator, or antihistamine medication as part of the treatment of other current disease states, and (c) a history of living 5 or more years in the Pittsburgh area. Only 100 of the first 136 subjects tested fulfilled all the above criteria. Of those who did fulfill these criteria, 51 were white, 49 Negro, 55 female, and 45 male. The age range was from 16 to 81 years, and 76 were over the age of 40.

From the Department of Medicine, University of Pittsburgh, Pittsburgh, Pa.
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*Senior Medical Student Fellow of the Allergy Foundation of America.
**Requests for reprints should be sent to R. S. Farr.

Antigens.—The antigens used in this experiment were prepared by the Allergy Laboratory, Montefiore Hospital, Pittsburgh, Pa. The pollen antigens were extracted from dry defatted pollens by incubation in Coca's solution* for 24 to 48 hours. After Seitz filtration, glycerin was added and the solutions were diluted with buffered saline† so that the most concentrated pollen extracts used for skin testing contained 0.1 mg. N per milliliter and 6.0 per cent glycerin. The dust and mold extracts were prepared in a similar fashion to the pollen extracts, except that no glycerin was added. The concentrated dust extract contained 0.009 mg. N per milliliter and the concentrated mold extract contained 0.015 mg. N per milliliter.

The five antigenic substances used in this study were extracts of ragweed, mixed trees, mixed grasses, mixed molds, and house dust; the two control solutions tested on each subject were glycerin-saline and histamine-saline. The concentrated ragweed antigens contained equal parts of extracts of tall ragweed and short ragweed with 6 per cent glycerin. The concentrated mixed tree antigens contained equal parts of extracts of maple, elm, poplar, hazelnut, birch, ash, hickory, black walnut, sycamore, oak, and beech with 6 per cent glycerin. The concentrated mixed grass antigens contained equal parts of extracts of timothy, orchard grass, redtop, June grass, sweet vernal, and perennial rye with 6 per cent glycerin. The concentrated mixed mold antigens contained equal parts of extracts of alternaria, hormodendrum, aspergillus, and penicillium. The concentrated dust extract was made from vacuum cleanings from carpets, pillows, mattresses, and upholstered furniture. The concentrated glycerin-saline control contained 6 per cent glycerin in buffered saline. The concentrated histamine control was a 1:1000 solution of histamine phosphate in buffered saline. Each of the seven concentrated test substances was diluted 1:10 and 1:100 in buffered saline.

Testing.—Subjects were first given intradermal injections of approximately 0.02 ml. of the 1:100 dilutions of the seven concentrated test solutions. Disposable plastic syringes with disposable No. 27 needles were used, the test sites were one inch apart on the lateral surface of the arm, and the results were read 15 minutes after injection. Based on the observations of Grow,[1] a wheal 5 mm. or greater in diameter was the preselected size considered to be a positive intradermal test. Erythema was not used as a criterion because it could not be evaluated on pigmented skin and 49 per cent of the subjects were of the Negro race. If a test with the 1:100 dilution of any particular extract was negative after 15 minutes, the subject was retested with the 1:10 dilution. Finally, if the 1:10 dilution was also negative for a given substance, the most concentrated test solution was applied. During the waiting period between these series of tests, details of the personal and family allergic history were obtained from each patient.

*Coca's solution contains 70 Gm. NaCl, 38.4 Gm. $NaHCO_3$, and 56 Gm. of phenol dissolved in 14,000 ml. of distilled water.

†The buffered saline contains 75 Gm. NaCl, 5.5 Gm. KH_2PO_4, 16 Gm. $Na_2HPO_4 \cdot 7H_2O$, and 58 Gm. phenol dissolved in 15,000 ml. of distilled water.

RESULTS

Pollen Extracts.—The data shown in Table I summarize the incidence of positive intradermal tests observed with each concentration of each antigen and each control solution. Most of the information contained in Table I is self-explanatory, but it is noteworthy that a positive test was obtained in 16 per cent of the subjects injected with the 6 per cent glycerin-saline control solution. That the 6 per cent glycerin was responsible for these positive reactions rather than the buffered saline is evidenced by the fact that none of the subjects responded to 0.6 or 0.06 per cent glycerin suspended in the same diluent. The data also suggest that positive reactions to the 6 per cent glycerin-saline solutions were on the basis of a nonspecific irritation because of the 16 subjects, who reacted to the 6 per cent glycerin-saline control solution, only 7 responded to all three antigens containing the same per cent of glycerin, only 12 responded to at least two of the antigens containing the same glycerin, 14 responded to at least one other of the concentrated antigens, and 2 responded to the 6 per cent glycerin-saline control solution alone. Had the glycerin response been specific, almost all of the subjects responding to glycerin in saline should also have responded to the same concentration of glycerin present in the ragweed, grass, and tree antigens.

TABLE I. INCIDENCE OF POSITIVE INTRADERMAL SKIN TESTS IN A POPULATION OF 100 INDIVIDUALS WITHOUT HISTORY OF RHINITIS AND/OR ASTHMA

	0.1 MG. N/ML. WITH 6% GLYCERIN	0.01 MG. N/ML. WITH 0.6% GLYCERIN	0.001 MG. N/ML. WITH 0.06% GLYCERIN
Ragweed with glycerin	28%	8%	0
Mixed grasses with glycerin	35%	2%	0
Mixed trees with glycerin	30%	3%	0
Saline diluent with glycerin	16%	0	0
	Conc.	1:10	1:100
Mixed molds without glycerin	17%	2%	1%
Dust extract without glycerin	50%	19%	4%
	1:1000	1:10,000	1:100,000
Histamine phosphate	100%	100%	92%

Regarding the results obtained with the dilute solutions of the grass, ragweed, and tree pollens, it is highly significant that none of the 100 control subjects responded with a positive test to the antigen containing only 0.001 mg. N per milliliter. These findings are in contrast to an incidence of approximately 85 per cent positive skin reactions when this concentration of this ragweed extract was tested on 66 subjects with a well-documented history of rhinitis and/or asthma during the ragweed season.[6]

Dust and Mold Extracts.—It can be seen in Table I that the dust extracts used in these studies caused more positive skin reactions than did the pollen or mold extracts. The positive reactions observed with glycerin are of no concern to the dust and mold extract results since these extracts contained no glycerin.

The Incidence of Multiple Positive Skin Tests.—The incidence of multiple positive skin tests to the five antigens and the glycerin diluent in the patients presented in Table I is summarized in Table II. It may or may not be significant that 5 of the 8 persons responding to 0.01 mg. N per milliliter of ragweed also responded to the 1:10 dilution of dust extract, but no other cross-reactivity patterns were noted with respect to skin testing with the antigens employed. It is apparent in Table II that the most concentrated extracts used for this study elicited too many positive reactions in asymptomatic individuals to be of much assistance as a diagnostic aid in the identification of offending substances in cases of rhinitis or asthma. Even the five 1:10 dilutions of the concentrated antigens elicited one or more reactions in almost 25 per cent of the patients. As mentioned above, reactions to the 1:100 dilution of the concentrated antigens were extremely uncommon in asymptomatic individuals and, therefore, a positive reaction to this dilute antigen becomes a highly significant observation when associated with a compatible clinical history.

TABLE II. THE INCIDENCE OF MULTIPLE POSITIVE SKIN TESTS TO THE FIVE ANTIGENS AND THE ANTIGEN DILUENTS IN THE PATIENT GROUP PRESENTED IN TABLE I

ANTIGEN OR DILUENT CONCENTRATIONS	1 OR MORE POSITIVE TESTS (%)	2 OR MORE POSITIVE TESTS (%)	3 OR MORE POSITIVE TESTS (%)	4 OR MORE POSITIVE TESTS (%)	5 OR MORE POSITIVE TESTS (%)	6 OR MORE POSITIVE TESTS (%)
Concentrated	71	48	25	19	11	2
1:10	24	5	3	3	0	0
1:100	4	1	0	0	0	0

Positive Intradermal Tests as Related to Other Factors.—An attempt was made to relate the incidence of skin sensitivity with the five test antigens to the past history of allergic manifestations other than rhinitis and asthma. Such a consideration was necessary because 46 of the subjects gave a history of having had intermittent hives, poison ivy, or drug reactions sometime in the past. As seen in Table III there was no correlation between such personal histories and skin sensitivity to the test antigens employed except in the case of subjects who previously had contact dermatitis. Of those having a personal history of contact dermatitis (primarily poison ivy), 95 per cent reacted to one or more of the concentrated antigens and 37 per cent reacted to one or more of the 1:10 antigen dilutions, but only 5 per cent reacted to the 1:100 dilution. These results may be significantly different from the data collected from individuals having no personal history of contact dermatitis, among whom only 65 per cent reacted to the concentrated antigen, only 21 per cent to the 1:10 dilution, and 4 per cent to the 1:100 antigen dilution. These data are in keeping with the previous suggestion that the skin of individuals having had contact dermatitis is hyperreactive to nonspecific irritation[7] since the post contact dermatitis group was not hyperreactive to the dilute solution of antigen where skin testing becomes highly specific.

The data relative to family history, race, sex, and age are summarized in Table IV. No correlation could be made between the occurrence of positive intradermal tests and a family history of hives, eczema, asthma, or hay fever. In

TABLE III. THE PER CENT OF ONE OR MORE POSITIVE SKIN TESTS IN PATIENTS WITH A HISTORY OF ALLERGIC CONDITIONS OTHER THAN RHINITIS AND/OR ASTHMA

	NO. OF CASES	1:100 ANTIGEN (%)	1:10 ANTIGEN (%)	CONC. ANTIGEN (%)
1. History of hives	28	4	21	64
No history of hives	72	4	25	74
2. History of contact dermatitis	19	5	37	95
No history of contact dermatitis	81	4	21	65
3. History of drug reactions	18	0	11	50
No history of drug reactions	82	5	27	76
4. History of either 1, 2, or 3	46	4	26	67
No history of 1, 2, or 3	54	4	21	74
5. Average of all patients	100	4	24	71

addition, there was no significant difference in the incidence of positive tests as related to age or race, but there was a rather marked tendency for males to have more positive tests to the lower concentrations of antigen than was observed among the females (Table IV). If significant, the reason for the difference is not clear.

TABLE IV. THE PER CENT OF ONE OR MORE POSITIVE SKIN TESTS AS RELATED TO SEX, RACE, AND FAMILY HISTORY OF ALLERGY

	NO. OF CASES	1:100 ANTIGEN (%)	1:10 ANTIGEN (%)	CONC. ANTIGEN (%)
1. Females	55	0	15	73
2. Males	45	9	36	69
3. Under age of 40	24	8	33	66
4. Over age of 40	76	3	21	72
5. White	51	6	24	63
6. Negro	49	2	24	80
7. With family history of allergy	19	0	16	53
8. Without family history of allergy	81	5	26	75
9. Average of all patients	100	4	24	71

Passive Transfer Studies.—The question arose as to whether the positive reactions observed with the intermediate concentrations of antigen were due to skin sensitizing antibody, or whether they were due to a nonspecific irritation effect. Accordingly, 0.1 ml. of serum from each of the 5 patients responding to both the 0.01 mg. N per milliliter of ragweed extract and to the 1:10 dilution of dust extract, and from each of the 3 patients reacting to the 0.01 mg. N per milliliter of ragweed alone, was injected intradermally into a normal recipient. Forty-eight hours after application to the normal recipient, all 8 sites were challenged with ragweed antigen containing 0.1 mg. N per milliliter without glycerin. Of the 8 sera tested, 3 were clearly shown to have passively transferable skin sensitivity antibody as evidenced by fully developed Prausnitz-Küstner (P-K) test reactions with wheals, pseudopodia, erythema, and itching. As controls for this experiment, 1:10,000 histamine applied to the recipient was positive, and three normal sites on the recipient were unequivocably negative to this concentration of ragweed extract.

Forty-eight hours after the recipient sites were challenged with ragweed extract, the same 8 sites were again challenged with the 1:10 dilution of dust extract. On this occasion, 4 of the 5 sera from patients with positive direct tests to the 1:10 dust extract had strongly positive reactions and all 3 of the sera from patients with negative direct tests were also negative to the P-K test. Three normal sites on the recipient did not respond to the 1:10 dilution of dust extract. Details of this experiment are presented in Table V, and it was concluded from these data that skin sensitizing antibody to dust or to ragweed was indeed present in 5 of the 8 sera tested.

DISCUSSION

It is not possible to compare directly the results obtained in this study with previous reports in the literature because (a) a variety of methods have been used to express the concentration of extracts used for testing, (b) the presence of multiple antigens in pollen extracts limits the significance of any attempt made to quantitate the amount of antigen in a given extract, and (c) it is highly unlikely that any two extracts prepared from dust or mold, or from plant, weed, or tree pollen would contain equal concentrations of the multiple antigens known to be present in each instance. In general, however, the data in Table I are in reasonable accord with the existing literature which describes the incidence of positive intradermal tests when the antigens and the concentrations of antigens similar to those used for this study were injected into asymptomatic individuals.[1-5] For example, Efron and Boatner[5] observed 5 per cent positive reactions with 0.002 per cent of their solution of "purified pollen extracts" and 10 per cent positive reaction with 0.02 per cent of the same ragweed antigen solution.

The incidence of positive and of negative intradermal tests, when antigens are injected into the skin of asymptomatic individuals, has considerable bearing upon the interpretation of results when the same antigens are tested on patients with problems involving rhinitis and asthma. For example, a positive reaction obtained with a 0.001 mg. N solution of the pollen extracts of trees, ragweed, and grasses used for this study becomes a highly significant clinical finding when a patient has physical manifestations of allergic disease during the season of the year when such antigens are known to be present in the air of the local environment. Conversely, a positive reaction to the most concentrated extracts used in this study are of little diagnostic aid when found to occur on the skin of a patient with perennial rhinitis or asthma of unknown etiology. When confronted with a patient with symptoms of unknown etiology, it is difficult to assign a casual relationship between a positive skin test with a concentrated antigen and the symptoms when approximately 50 per cent of an asymptomatic population have two or more positive reactions when tested with a battery of only five of the concentrated antigen solutions.

The demonstration of passively transferable antibody in the sera of 5 of the 8 positive reactors to the intermediate concentrations of ragweed and dust indicates that at least 5 per cent of the asymptomatic individuals studied had

TABLE V. PASSIVE TRANSFER STUDIES ON THE SERA FROM 5 ASYMPTOMATIC INDIVIDUALS WITH POSITIVE INTRADERMAL TESTS TO RAGWEED AND DUST AND 3 ASYMPTOMATIC INDIVIDUALS WITH A POSITIVE INTRADERMAL TEST TO RAGWEED ALONE

	PATIENT							
	C. Mc	M. T.	P. M. P.	K. M.	N. M.	F. I. J.	W. G.	C. L. G.
Age	25	60	24	66	64	54	74	35
Sex	F	M	M	M	F	M	M	M
Race	W	W	W	W	N	W	N	N
Diagnosis	Rheumatic heart disease	Essential hypertension	Normal student	Degenerative joint disease	Normal housewife	Diabetes mellitus	Peptic ulcer	Malignant hypertension
Reaction recorded*	Wheal; erythema	Wheal; erythema	Wheal; erythema	Wheal; erythema	Wheal	Wheal; erythema	Wheal	Wheal
Direct test 0.6% glycerin (control)	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Direct test ragweed (0.01 mg. N/ml.)	5 × 5 mm. + P† 15 × 15 mm.	5 × 5 mm. 20 × 20 mm.	5 × 7 mm. 25 × 25 mm.	5 × 6 mm. 0	6 × 6 mm.	6 × 6 mm. 20 × 20 mm.	5 × 5 mm.	5 × 5 mm.
P-K test ragweed (0.1 mg. N/ml.)	8 × 8 mm. + P 30 × 30 mm.	10 × 7 mm. + P 35 × 35 mm.	Neg.	Neg.	6 × 6 mm. 25 × 25 mm.	Neg.	Neg.	Neg.
Direct test (1:10 dust)	5 × 6 mm. + P 15 × 20 mm.	6 × 6 mm. 20 × 20 mm.	7 × 6 mm. 30 × 30 mm.	7 × 7 mm. 10 × 10	7 × 7 mm. + P	Neg.	Neg.	Neg.
P-K test (1:10 dust)	8 × 8 + P 25 × 25 mm.	9 × 7 mm. + P 30 × 30 mm.	7 × 6 mm. 15 × 15 mm.	7 × 7 mm. 20 × 20 mm.	Neg.	Neg.	Neg.	Neg.

*Erythema not recorded on pigmented skin.
†P = pseudopodia.

positive intradermal tests associated with the presence of skin sensitizing antibody in their sera. The 5 per cent incidence indicated is undoubtedly an underestimate since the passive transfer studies were not done on all sera from positive reactors. The other sera were not tested because of an unwillingness to run the additional risks of inoculating recipients with the B virus responsible for serum hepatitis. If one assumes that the incidence of positive P-K tests would have been approximately the same with the 16 additional sera from subjects who had a direct positive test to the 0.01 mg. N per milliliter of pollen extracts, or the 1:10 dust or mold extracts, then it would appear that detectable amounts of skin sensitizing antibody against one or more of the five antigens were present in the sera of over 10 per cent of the asymptomatic individuals in this study.

Regarding nonspecific irritation as a cause for positive tests, some of the weak responses in the ragweed group may well have been caused by a nonspecific irritation. In addition, 16 per cent of the subjects reacted to the 6 per cent glycerin-saline control solution. That these reactions were also nonspecific is suggested by the fact that only 7 of the 16 subjects reacted to all three of the antigens containing the same concentration of glycerin. It is probable that the converse of this situation also occurred in that some patients must have responded to the irritating effects of 6 per cent glycerin in one or more of the concentrated antigens at a time when the test with the 6 per cent glycerin control solution was negative. These findings are probably best interpreted as an indication that many nonspecific positive reactions occurred when test antigens containing 6 per cent glycerin were employed, but that some of the positive reactions to the concentrated pollen, mold, and dust extracts may also have been on the basis of a specific antigen-antibody interaction.

The clinical significance of a positive test due to specific antigen-antibody interaction on the skin of an asymptomatic individual is not entirely clear. Chambers and Glaser[8] and Ratner[9] present convincing evidence that some of the asymptomatic individuals, particularly children, with positive skin tests to ragweed pollen develop clinical symptoms of seasonal pollinosis in subsequent years. On the other hand, as pointed out by Feinberg[10] and by Fuchs,[11] it is most unusual that an individual will develop clinical symptoms of seasonal pollinosis after the age of 40. In the present study, 67 of the positive results obtained with the 1:10 and 1:100 dilutions of the concentrated antigens were elicited on the skin of individuals older than 40. Since many of the positive results with the 1:10 and 1:100 dilutions of antigen were probably due to specific antigen-antibody interactions, it follows that most of the older age group individuals with positive tests will not develop seasonal pollinosis in the future despite the fact that their sera contained skin sensitizing antibody. This complete lack of correlation between positive intradermal tests and clinical symptoms is not surprising in view of the fact that a high degree of skin reactivity can and frequently does remain when clinically sensitive patients become asymptomatic as a result of specific hyposensitization.[12] A striking example of this situation was recently observed in a patient who had severe symptoms of ragweed pollinosis for 3 years prior to becoming completely asymptomatic without

specific therapy. At the end of his second asymptomatic season, this individual had a 4+ reaction to 0.0001 mg. N per milliliter of the ragweed antigens used for the present tests.[13]

The present experiments indicate that skin sensitizing antibody can be detected in the sera of a relatively large number of individuals who do not have clinical manifestations of asthma or rhinitis. It is equally true that detectable skin sensitizing antibody to ragweed, for example, is usually present in the serum of a patient with rhinitis or asthma during the hay fever season. Although it seems likely that skin sensitizing antibody must be present in order for a patient to have symptoms of seasonal pollinosis, it is also evident that skin sensitizing antibody can be present without clinical manifestations of allergy. This relationship between the presence of antibody and clinical manifestations of allergy is similar to the experimental allergic fevers produced in rabbits immunized with crystalline bovine serum albumin (BSA).[14, 15] In this experimental model: (a) normal rabbits do not respond with fever when challenged with intravenous BSA, (b) about half of a group of rabbits immunized with BSA respond with fever when challenged with intravenous BSA, (c) some of the nonresponding rabbits have more circulating anti-BSA than those who do respond with fever, and (d) the capacity to respond with fever following intravenous administration of BSA can be passively transferred to normal rabbits by means of serum, but not by means of cells.[16, 17] Thus, antibody is probably necessary for both the fever reaction in rabbits and for seasonal pollinosis symptoms in man, but fever and pollinosis do not necessarily result when antibody is present.

CONCLUSIONS

1. The incidence of positive intradermal reactions has been studied when 100 asymptomatic individuals were tested with graded concentrations of each of five antigen extracts.

2. The incidence of positive reactions to these antigens ranged from 50 to 0 per cent, depending upon the antigen and the concentration of extract used.

3. Six per cent glycerin, a commonly used stabilizer of skin test antigens, elicited positive intradermal tests in 16 per cent of the individuals tested; 0.6 per cent glycerin in saline did not elicit any positive intradermal tests.

4. At least 5 per cent of the asymptomatic individuals studied had positive intradermal tests associated with the presence of skin sensitizing antibody in their sera. The 5 per cent incidence is undoubtedly an underestimate since the passive transfer studies were attempted with only 8 sera.

5. Positive intradermal tests and passively transferable skin sensitizing antibody against ragweed and dust were found in some individuals older than 40 years of age and who are probably destined to remain asymptomatic for the rest of their lives.

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GOITER AND MYXEDEMA FROM IODINE

Irvin Caplin, M.D., George F. Parker, M.D.,** Jack H. Hall, M.D.,*** and Hooshang Khajezadeh, M.D.,**** Indianapolis, Ind.*

GOITER resulting from iodine administration is an entity which no longer can be considered rare. It is our belief that a review of patients in any clinic or practice where large numbers of asthmatics are treated with iodine would result in the discovery of many unreported cases. It is an entity easily recognized and simply treated. The association of iodine and thyroid disease has been known since the discovery of the element early in the nineteenth century. Chatin,[1] in 1850, carried out some of the first scientific investigations into the relationship between iodine and goiter, and showed that endemic goiter areas had low content of iodine present in the water and soil. In 1909, Marine and Lenhart[2] called attention to the fact that iodine deficiency resulted in goiter. In 1923, Plummer[3] described the suppressive effect of iodine on thyroid overactivity in exophthalmic goiter. In 1928, Haines[4] noted that iodine therapy of hyperthyroidism might lead to myxedema. In 1945, Hurxthal[5] described myxedema and increased goiter after the treatment of a simple goiter with iodine.

The occurrence of goiter and myxedema in patients with apparently normal thyroid glands, while on iodine therapy, was first reported by Bell[6] in 1952. He described three asthma patients who developed goiter and myxedema after prolonged iodine administration. Morgans and Trotter[7] in 1953 independently observed 2 cases of myxedema attributed to iodine administration. They believed that one reason previous reports of myxedema from iodine had not appeared in the literature is that a diagnosis of myxedema is usually automatically followed by the administration of thyroid extract without a search for a possible causative agent. Falliers,[8] in April, 1960, had found reports of a total of 29 cases of goiter, with or without myxedema, which were attributable to the ingestion of large amounts of iodine and 27 of these patients were asthmatics. He added 4 new cases, in all of which the patients had asthma. In June, 1960, Paris and associates[9] reported on 11 patients with iodine goiter seen at the Mayo Clinic since 1956. Of these 11, 3 were asthmatics.

The following 3 cases studied at the Methodist Hospital Allergy Clinic, since 1959, present the same typical patterns of goiter with or without myxedema occurring during prolonged iodine therapy.

From The Methodist Hospital Graduate Medical Center, Indianapolis, Ind.
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*Director of Allergy Clinic, Methodist Hospital Graduate Medical Center.
**Associate Director of Allergy Clinic, Methodist Hospital Graduate Medical Center.
***Director of Medical Education, Methodist Hospital Graduate Medical Center.
****Chief Resident in Pediatrics, Methodist Hospital Graduate Medical Center.

CASE REPORTS

CASE 1.—J. S., a 31-year-old white man, was first seen in January of 1956 and treated for perennial asthma which he had had since the age of 3. On July 21, 1959, he was seen with complaints of swelling of the face of about 10 days' duration, body numbness and tingling, tiredness, weakness, and a weight gain of about five and one-half pounds. For the previous 2 years he had been taking one "Synate" tablet at bedtime. Each tablet of "Synate" contains: theophylline–sodium glycinate, 360 mg.; racephedrine hydrochloride, 30 mg.; potassium iodide, 300 mg.; secobarbital, 20 mg.; and niacinamide, 40 mg.

Physical examination revealed a dry skin and a rounded face. There appeared to be a slight ptosis of the eyelids. The thyroid gland, which had not been palpable prior to this examination, was diffusely enlarged. The blood pressure was 100 systolic and 80 diastolic. The heart rate was 58 beats per minute. The Achilles tendon reflex was slow. The hemogram and urinalysis results were normal. The basal metabolic rate (BMR) on two occasions was −34 and −37. The serum cholesterol was 357 mg. per 100 ml. An electrocardiogram revealed a decrease in voltage and a flattening of the T waves throughout all leads when compared to a previous tracing. The iodine-containing "Synate" medication was discontinued and 30 mg. of desiccated thyroid extract was administered daily. After 10 days, examination revealed a loss of three pounds of weight and less rounding of the facies. The thyroid gland remained enlarged. The patient felt much more alert and vigorous. His thyroid dosage was reduced to 15 mg. every other day for 2 weeks and was then discontinued. He continued to improve. In 1 month, all evidence of myxedema had disappeared. The goiter slowly became smaller and required 3 months to disappear.

CASE 2.—D. H., a 13-year-old white boy, had had severe perennial asthma for 10 years. When first seen 1 year ago in our clinic, he had severe asthma and a pigeon breast deformity. The thyroid gland was not palpable. As part of his symptomatic care, he was given 10 drops of saturated solution of potassium iodide four times daily.

After 11 months of this therapy, a diffuse enlargement of the thyroid gland was of sufficient degree to cause complaints of difficulty with swallowing. It was estimated that the thyroid gland would weigh 75 grams (normal 25 to 30 grams). There was also exophthalmos. The BMR was −12 and −7. The butanol extractable iodine (BEI) was 3.2 mcg. per 100 ml. (normal, 3.6 to 5 mcg. per 100 ml.).

The iodine was discontinued. Although the goiter gradually became smaller, it was still palpable at the end of 5 months. Sea food and iodized salt were eliminated from his diet. In 2 months there was a further reduction in the size of his goiter, although it did not completely disappear. The exophthalmos has persisted.

Recently, he developed status asthmaticus. Potassium iodide therapy was reinstituted along with desiccated thyroid. To date this has been successful. There has been no further increase in the exophthalmos or the size of his goiter.

CASE 3.—J. H., a 13-year-old white girl, had had severe perennial asthma for 11 years. In March of 1958, she was started on 10 drops of a saturated solution of potassium iodide four times daily. After 9 months, a diffuse enlargement of the thyroid was noted which had not been present previously. The gland was estimated to weigh approximately 60 grams. The serum cholesterol was 218 per 100 ml., and the BEI was 4.2 mcg. per 100 ml., both of which are normal. She did not appear myxedematous. The iodides and iodine-containing foods and salt were eliminated. The goiter disappeared over a period of 7 months.

DISCUSSION

Bell,[6] in his original report in 1952, quoted Marine and Lenhart[2] who stated that iodine deficiency is generally accepted as the principal cause of endemic or colloid goiter. Iodine deficiency goiters may be due to inadequate iodine intake,

increased demands for thyroid substance, failure of the thyroid gland to utilize normal iodine supply (as occurs in certain goitrous cretins), ingestion of certain antithyroid substances, such as aminopyrine, cyanogens, and the thiouracil drugs.

Bell also noted that iodine in large amounts does not reduce the size of a goiter already present, although iodine in minute amounts usually prevents goiter formation. He quoted evidence suggesting that the synthesis of thyroid hormone may be suppressed by high doses of iodine by inhibiting the organic binding of iodine by the thyroid gland. He also noted that it had been shown that iodine suppresses the release of thyroid hormone by inhibiting partially, at least, the proteolytic enzyme system which participates in the release of thyroxine from thyroglobulin.

Gold[10] stated that most authors believe that iodine blocks the synthesis of thyroxine within the thyroid gland, thus leading to hypothyroidism and myxedema. This decrease in circulating thyroxine results in the overproduction of thyroid stimulating hormone (TSH) by the pituitary which produces the goiter. He also described a microscopic finding of a papillary hyperplasia in the iodine goiter. He feels that this is the result of stimulation by the excess of the TSH.

Paris and co-authors[9] in their review believed that evidence suggested that the small number of patients who develop goiter from excess iodine must be unusually susceptible to the inhibitory effect of administered iodine on the thyroid gland's ability to bind iodine. This action is similar to that seen in the effect of iodine on exophthalmic goiter. Biopsy of 5 of their 11 patients revealed parenchymatous infiltration. They stress the fact that Hashimoto's thyroiditis must be considered in the differential diagnosis of "iodine goiter." Clinically the two can be similar.

Burrows and his associates[11] reported that their fourth case of iodine goiter had come to operation. The cause of the goiter was not recognized and a subtotal thyroidectomy was done. Microscopic examination revealed a typical colloid goiter which is in variance with the papillary hyperplasia reported by Gold.

This entity of goiter produced by iodine ingestion must be considered in a differential diagnosis of goiters. One should be suspicious of iodine being a related factor in an allergic patient, or a patient who gives a history of prolonged ingestion of cough syrups or iodides. The diagnosis is established when on the complete withdrawal of iodine the myxedema disappears and the goiter slowly becomes smaller. The myxedema usually clears rapidly, often in the first week after iodine is withdrawn; the goiter may take 7 or 8 months to disappear or may remain palpable, as in our second case.

Case 2 illustrated that when iodine administration is imperative in the case of an iodine goiter and myxedema, the patient may be given thyroid extract along with iodine. The thyroid extract prevents myxedema and subsequent goiter formation.

Burrows and co-workers[11] observed that most euthyroid patients exhibited no thyroid derangement while taking iodine. Goodman and Gilman[12] state that the normal thyroid gland is uninfluenced by iodine. This is no doubt true, as

those who do develop iodine goiters are few in comparison to the number of patients who take large doses of iodine over prolonged periods of time. The similarity between patients with myxedema and goiter from iodine and patients with Graves' disease, when iodine is administered, is striking. It is possible that these patients have a subclinical derangement of thyroid function which becomes perceptible after excessive or prolonged iodine administration. In Graves' disease, iodine apparently blocks the release of effective thyroid hormone from the gland. Perhaps this same mechanism produces the iodine myxedema and goiter. Management of these patients is simple. When iodine is discontinued, the myxedema usually disappears in several days to several weeks; the goiter may take months (7 in our Case 3) to disappear, and may remain palpable and not disappear. It is important to recognize this entity and avoid unnecessary surgery. In patients whose asthma is of such severity that the continuation of iodine therapy is imperative, thyroid may be administered along with the iodine, as was done in our Case 2.

SUMMARY

Three cases are added to the growing list of goiters due to prolonged iodine administration; 1 of these patients also had myxedema. These patients seem to have an unusual sensitivity to the inhibitory effect of iodine on the production of effective thyroid hormone, either within the thyroid gland or its release from the thyroid gland. This mechanism is reversible by complete withdrawal of the iodine. The myxedema disappears within days to weeks; the goiter slowly becomes smaller over a period of many months and occasionally persists.

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SPECIFIC IMMUNE TOLERANCE TO ANAPHYLACTIC SENSITIZATION (EGG ALBUMIN) INDUCED IN THE GUINEA PIG BY CYCLOPHOSPHORAMIDE (CYTOXAN)

Henry C. Maguire, Jr., M.D., and Howard I. Maibach, M.D., Philadelphia, Pa.

SERUM antibody production following primary antigenic stimulation in the rabbit can be markedly inhibited by a suitably timed course of one of a number of cancer chemotherapeutic agents including 6-mercaptopurine (6-MP), nitrogen mustard, and x-ray.[1, 2, 3] Additionally, Schwartz and Dameshek[4] showed that the single course of 6-MP, which blocks the primary antibody response, specifically blocks further attempts to stimulate antibody formation to the particular antigen, but not to an unrelated antigen. They demonstrated this effect in the rabbit using bovine serum albumin as experimental antigen and measuring antibody formation with the I^{131} albumin disappearance technique of Talmadge and his co-workers.

We have studied the induction by cyclophosphoramide (Cytoxan)* of a long lasting immunologic unresponsiveness in the guinea pig.

Cyclophosphoramide is a nitrogen mustard derivative recently synthesized and found useful as a cancer chemotherapeutic agent. In a previous study we found that pretreatment with cyclophosphoramide blocked the formation of anaphylactic antibodies to egg albumin in the guinea pig.[5] The dosage schedule in the present experiment was essentially the same.

MATERIALS AND METHOD

Stock guinea pigs, weighing about 400 grams, were given intraperitoneal injections of cyclophosphoramide, 10 mg. per day for 8 days. Twenty milligrams of egg albumin in saline solution was given intraperitoneally 5 to 6 hours after the second cyclophosphoramide injection. By the sixth day of cyclophosphoramide treatment there was a marked depression of circulating leukocytes from the normal of 10,000 to less than 1,000. During this time, one fourth of the animals died and the remainder showed a moderate to marked morbidity.

From the Department of Dermatology of the University of Pennsylvania, Philadelphia, Pa.

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After the cyclophosphoramide was stopped, recovery took place rapidly and, by 2 weeks, all of the surviving guinea pigs were clinically and hematologically sound.

Twelve weeks after the original course of cyclophosphoramide and egg albumin the survivors were given another intraperitoneal injection of 20 mg. of egg albumin (no cyclophosphoramide). A control group of 12 guinea pigs from the original stock were also given the same intraperitoneal injection of egg albumin. Twenty-three days later the animals of both groups were challenged via an intracardiac injection of 4 mg. of egg albumin. Lethal anaphylaxis provided a sharp end point (Table I).

TABLE I

	TOTAL NO. ANIMALS	LETHAL ANAPHYLAXIS	SURVIVORS
Experimental group (egg Cytoxan pretreatment)	15	4	11
Control group	11	10	1

DISCUSSION

Anaphylactic death of 10 of 11 of the control group agrees with data in the literature and with comparable control groups for other experiments in our laboratory.[6] The guinea pigs pretreated with cyclophosphoramide and egg albumin 15 weeks earlier were distinctly less susceptible to anaphylactic sensitization by egg albumin. Generally, the re-treated animals that were protected showed no signs of anaphylaxis whatsoever; as a group they differed qualitatively from the control animals. The 11 survivors of the experimental group were subsequently sensitized to horse sera and upon elicitation exhibited 100 per cent lethal anaphylaxis.

It would seem that at least two agents—6 mercaptopurine and cyclophosphoramide—can produce a state of specific acquired immune tolerance of serum antibody production under the appropriate circumstances in mature animals. This drug-induced tolerance resembles the specific immune tolerance which arises from the administration of antigen to the newborn or unborn of suitable species; both situations require the presentation of specific antigen to a temporarily incompetent immune apparatus. As to what basic mechanisms may be involved, the atmosphere of consistent fact is at present both too rarified and turbulent to encourage speculative flights.

SUMMARY

An intraperitoneal injection of egg albumin along with the single course of a nitrogen mustard (cyclophosphoramide) was given to guinea pigs. Following a rest period of 3 months, a routine anaphylactic sensitization and challenge program resulted in 73 per cent survival in the experimental (pretreated) group and 9 per cent survival in the control group.

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IMMUNOLOGIC STUDIES OF RAGWEED-SENSITIVE PATIENTS TREATED BY A SINGLE REPOSITORY ANTIGEN INJECTION

Pierre J. Delorme, M.D., ** Maxwell Richter, Ph.D., Sheila Grant, M.Sc., Herbert Blumer, M.D.,* Arthur Leznoff, M.D., and Bram Rose, M.D., Montreal, Quebec*

THE treatment of allergic rhinitis and asthma due to pollens by the injection of allergens emulsified in oil has been attempted in the past on several occasions. However, the results were not very encouraging.[1, 2] It is only recently[3, 4, 5] that practical and useful methods of treatment, based on the emulsion principle, have been devised. Although this mode of treatment is now widely used, several pertinent questions remain unanswered, such as the utilization of objective methods which might demonstrate the efficiency or superiority of such treatment over the conventional method.[6] This paper is primarily concerned with immunologic studies (SSA† titers, blocking antibody titers, hemagglutinin titers) on the sera of 35 ragweed-sensitive patients who were all treated by a single repository ragweed injection in June, 1960, at the Royal Victoria Hospital Allergy Clinic.

METHODS AND MATERIALS

1. Selection of Patients.—Only patients who had clear-cut histories of ragweed sensitivity with positive skin tests to ragweed were included in this study. Most of these patients had been treated in 1959 by the conventional method (multiple injections of aqueous solutions of WSR‡) but all immunization injections had been terminated by August, 1959.

2. Repository Treatment.—The material used for preparing the emulsions was provided by the American Academy of Allergy as part of a large scale survey on this form of therapy. The mixture contains, in its oil phase, 9 parts of Drakeol 6 VR white mineral oil to 1 part of specially treated Arlacel A. The aqueous phase contains equal amounts of the water soluble extracts of defatted short and long ragweed pollen. Merthiolate (1: 10,000) was incorporated as a

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*Fellow of the National Research Council, Ottawa, Ontario.

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†SSA = Skin sensitizing antibody referred to generally as reagin.

‡WSR = Water soluble extract of ragweed pollen.

preservative. Two parts of the oil phase were used for each part of the aqueous phase. The emulsion was prepared by hand, using two Luer-Lock syringes with a double hub needle. The dosage used in each case was governed by the clinical sensitivity of the patient and by the titration of his skin reactivity with varying concentrations of a saline solution of WSR. Details of the method used in this study are outlined in a recent article by Feinberg and associates.[7] All injections were given by the intramuscular route in the latter part of June and in the early part of July. In those cases in which a local or a systemic reaction was observed following the repository injection, the reactions were graded as follows: local reactions at the site of injection were graded from 1+ to 4+, according to the degree of erythema, edema, and amount of discomfort present. Systemic reactions were also graded from 1+ to 4+. Mild symptoms of allergic rhinitis or generalized pruritus after the injection were classified as a 1+ reaction. Reactions characterized by either generalized urticaria and/or angioedema and/or asthma and/or severe gastrointestinal disturbances were classified as 2+, 3+, or 4+, according to the severity of those symptoms. After the ragweed season, the degree of subjective clinical improvement obtained by each patient was recorded as either complete, marked, moderate, fair, or none.

3. Immunologic Studies.—Blood was drawn from the patient before the repository injection was administered and 2½ months later when the ragweed season had ended. The blood was allowed to clot at room temperature for a few hours and then in the cold room at 4° C. overnight. The next day the serum, obtained by centrifugation, was frozen and stored in a deep freeze. Wassermann tests were performed on each post-treatment serum and a careful inquiry as to the possibility of recent or past history of hepatitis was done in each case. Pre- and post-treatment sera were always analyzed simultaneously. In each determination the end point titer is expressed as the reciprocal of the highest dilution of serum still giving a definite positive reaction. All ragweed or serum solutions used for passive transfer tests were made up in saline (0.9 per cent NaCl).

A. *Hemagglutinin titer determination:* The BDB (bis-diazotized benzidine) technique, as described by Gordon, Rose, and Sehon,[8] was used with the following modification: Neither the normal rabbit serum (used in the preparation of the "diluent" at a concentration of 1:100 in phosphate buffer) nor the human serum to be tested was decomplemented. The human serum was absorbed by the addition of an equal volume of packed rabbit erythrocytes and permitted to stand for 15 minutes at 4° C. The probability of obtaining hemolysis of the red cells because of the presence of complement precluded the use of room temperature for absorption. The red cells were then removed by centrifugation and discarded. The same amount of packed washed rabbit red cells was then added to the human serum again and absorption was allowed to proceed for another 15 minutes at room temperature. The red cells were then centrifuged off and discarded. With his modification of the absorption procedure we had no or only slight hemolysis, and nonspecific agglutination of the rabbit red cells by the human serum never occurred in the subsequent steps of the technique. We

also found that the slight hemolysis present in certain cases did not interfere with the normal course of the experiment. A single control serum of known titer was always run with each experiment.

B. Skin-sensitizing antibody (SSA) titer determination: The classical passive transfer method of Prausnitz and Kustner was used. On day 1, 0.05 c.c. of different concentrations of the human serum to be tested were injected into the skin of a recipient who was not sensitive to ragweed. The dilutions of the serum used were: 1:10, 1:25, 1:50, 1:125, 1:250, 1:500, 1:1000, 1:3000, and 1:6000. Twenty-four hours later, 0.05 c.c. of a saline solution of WSR containing 2,000 PNU* per cubic centimeter was injected into each site. The reaction was read after 20 minutes. The last definite positive reaction was recorded as the end point titer.

C. Blocking antibody titer determination: A "standard" serum obtained from a ragweed-sensitive subject was used as a constant source of reagin. By preliminary studies, using the passive transfer technique, it was found that the "reaginic activity" of 0.05 c.c. of a 1:100 dilution of this particular serum was completely neutralized by 0.05 c.c. of a saline solution of WSR containing 15 PNU per cubic centimeter. Each serum to be tested for blocking antibodies was heated at 56° C. for 1 to 2 hours in order to inactivate the SSA. The absence of skin-sensitizing activity after heating was demonstrated by passive transfer. It was found that some sera had to be heated for 2 hours to completely destroy the reaginic activity.

The blocking experiment was carried out in the following manner. On day 1, multiple sites on the skin of a suitable recipient were injected with 0.05 c.c. of a 1:100 dilution of the standard serum. On day 2, one cubic centimeter aliquots of serial dilutions (undiluted, 1:2.5, 1:5, 1:10, 1:15, 1:20, 1:30) of the unknown heated serum to be tested for blocking activity were incubated at room temperature for at least one hour with one cubic centimeter volumes of a solution of WSR in saline containing 30 PNU per cubic centimeter, so that the final dilutions of the serum would be 1:2, 1:5, 1:10, 1:20, 1:30, 1:40, and 1:60, and the final concentration of WSR in the mixture would be 15 PNU per cubic centimeter. At this point, 0.05 c.c. of each of these mixtures was injected into each site. On day 3, each site was reinjected with 0.05 c.c. of a solution of WSR containing 2,000 PNU per cubic centimeter. The reactions were read after 20 minutes. The reciprocal of the dilution of the unknown serum in the site giving the last definite positive reaction was recorded as the blocking antibody titer.

RESULTS

1. Changes in Hemagglutinin Titer.—Twenty-two out of the 35 sera had an increase in hemagglutinin titer of fourfold or more. Eight of the sera exhibited a twofold increase in titer and in 5 the titers remained the same (Table I). No decrease in titer was observed. Neither the amount of ragweed given, the

*PNU = Protein nitrogen unit.

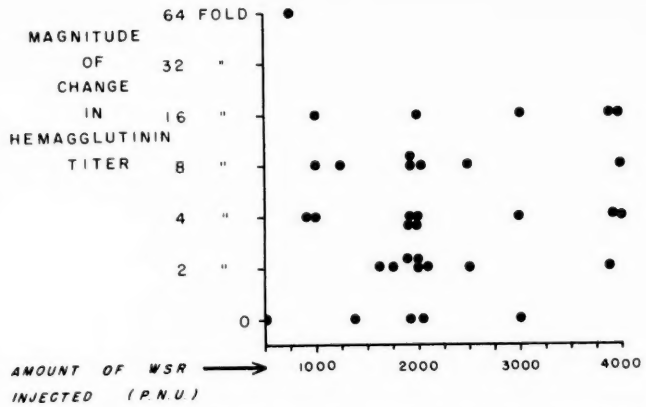

Fig. 1.—Relation between the amount of ragweed injected and the magnitude of change in hemagglutinin titer (35 cases).

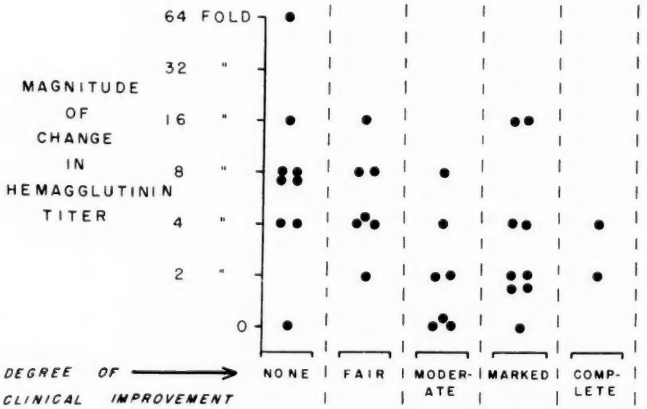

Fig. 2.—Relation between the degree of clinical improvement and the magnitude of change in hemagglutinin titer. (34 cases).

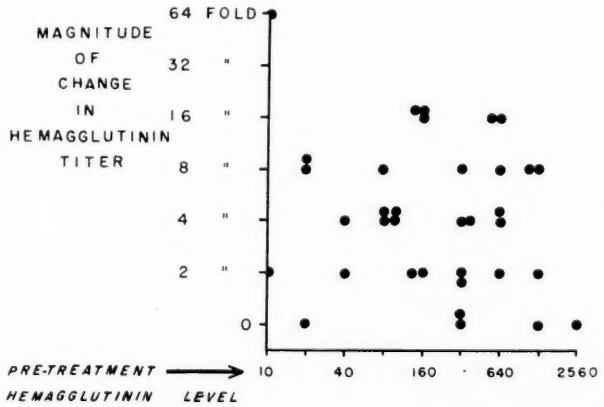

Fig. 3.—Relation between the magnitude of change in hemagglutinin titer and the pre-treatment hemagglutinin level (35 cases).

degree of subjective clinical improvement, nor the pre-treatment hemagglutinin level could be correlated with the magnitude of change in titer (Figs. 1, 2, and 3).

2. Changes in Skin Sensitizing Antibody (SSA) Titer.—SSA titers were determined in 31 cases, the remaining 4 patients having had a history of past or recent hepatitis. An increase in titer was considered significant only when there was more than a twofold difference between the pre- and post-treatment sera. Eight patients had an increase in SSA titer after repository therapy, which was accompanied in all by an increase in hemagglutinin titer. In 12

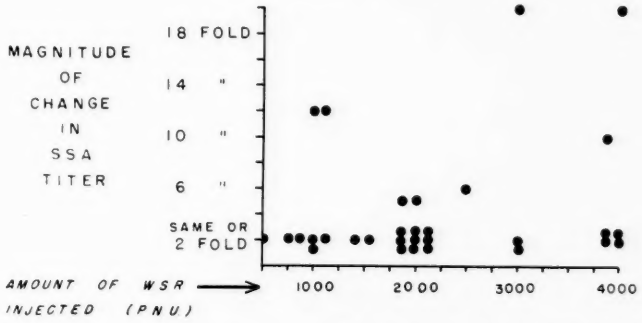

Fig. 4.—Relation between the amount of ragweed injected and the change in SSA titer (31 cases).

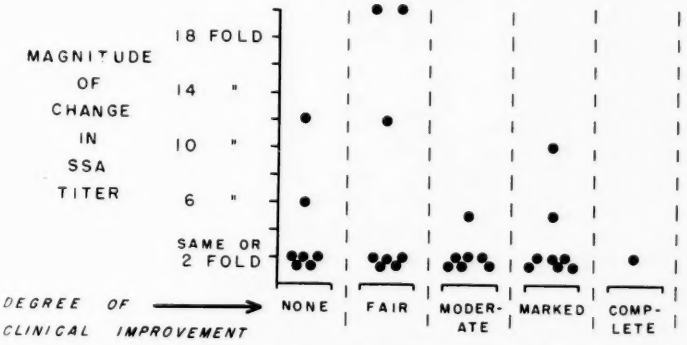

Fig. 5.—Relation between the degree of clinical improvement and the change in SSA titer (31 cases).

cases, however, in spite of a rise in hemagglutinin level, the SSA titer remained unchanged. In 11 sera, no variation in SSA titers was observed and there was either no change or a twofold increase in hemagglutinin titer. A twofold increase in hemagglutinin titer is not considered significant (Table II.) A decrease in SSA titer after treatment was not observed in any serum. No relationship could be established between the variations in SSA titer and the degree of subjective clinical improvement or the amount of ragweed injected (Figs. 4 and 5).

3. Changes in Blocking Antibody Titer.—Blocking antibody titer determinations were performed in 12 cases. Four typical samples were chosen from each group outlined in Table II. As can be seen in Table III, changes in

TABLE I. CHANGES IN HEMAGGLUTININ TITER AFTER REPOSITORY THERAPY (35 CASES)

MAGNITUDE OF CHANGE	NO. OF PATIENTS
0	5
2-fold	8
4-fold	9
8-fold	6
16-fold	6
32-fold	0
64-fold	1

TABLE II. VARIATIONS IN SSA AND HEMAGGLUTININ TITERS (31 CASES)

	NO. OF CASES
Group I Hemagglutinins increased—SSA increased	12
Group II Hemagglutinins increased—SSA no change	8
Group III Hemagglutinins no change—SSA no change	11

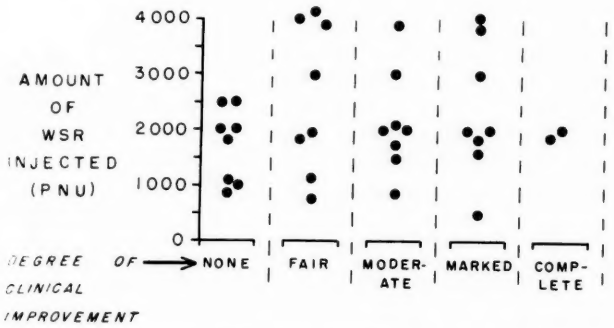

Fig. 6.—Relation between the degree of clinical improvement and the amount of ragweed injected (34 cases).

blocking antibody titers bear no relationship to either the variations in hemagglutinin levels, the changes in SSA titers, the amounts of ragweed given, or the degree of subjective clinical improvement.

TABLE III. BLOCKING ANTIBODY, HEMAGGLUTININ AND SSA TITERS, AMOUNT OF RAGWEED GIVEN, AND DEGREE OF CLINICAL IMPROVEMENT (12 CASES)

GROUP AND PATIENT	HEMAGGLUTININ TITER		SKIN-SENSITIZING ANTIBODY TITER		BLOCKING ANTIBODY TITER		AMOUNT OF RAGWEED GIVEN (PNU)	DEGREE OF IMPROVEMENT
	PRE	POST	PRE	POST	PRE	POST		
Group I								
1	640	10,240	50	1,000	20	30	3,000	Fair
2	1,280	10,240	50	1,000	30	40	4,000	Fair
3	80	640	500	6,000	0	10	1,100	Fair
4	20	320	10	50	0	10	1,935	Marked
Group II								
5	640	10,240	1,000	1,000	5	20	3,870	Fair
6	1,280	10,240	500	1,000	0	0	1,935	None
7	40	160	50	25	10	30	3,000	Marked
8	80	320	1,000	1,000	0	20	1,935	None
Group III								
9	20	20	1,000	1,000	2	10	500	Marked
10	320	320	1,000	1,000	0	0	1,400	Moderate
11	2,560	2,560	500	1,000	0	0	1,935	Moderate
12	1,280	1,280	500	500	30	30	3,000	Moderate

4. Relationship Between the Amount of Ragweed Given and the Degree of Subjective Clinical Improvement.—No correlation appears to exist between the amount of ragweed given and the degree of subjective clinical improvement obtained (Fig. 6).

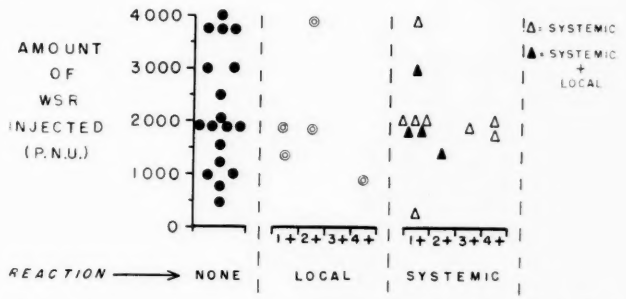

Fig. 7.—Relation between the amount of ragweed given and the degree of local or systemic reaction following repository injection (35 cases).

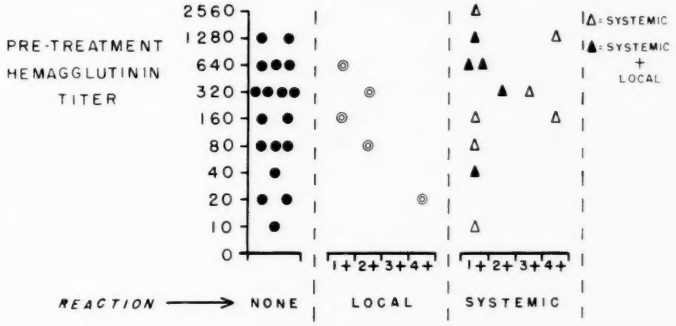

Fig. 8.—Relation between the pre-treatment hemagglutinin titer and the degree of local or systemic reaction following repository injection (35 cases).

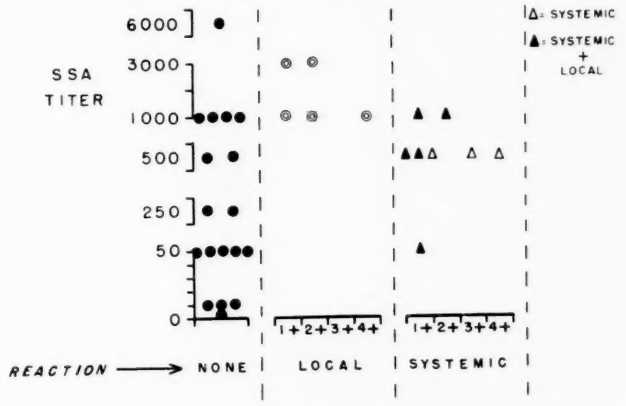

Fig. 9.—Relation between the pre-treatment SSA titer and the degree of local or systemic reaction following repository injection (31 cases).

5. Reactions Following Repository Injection.—A systemic or a local reaction after the repository injection was observed in 17 out of 35 cases. Twelve patients had systemic reactions but these reactions were severe in only 3. The incidence of reactions or the degree of severity of these reactions could not be related to

the amount of ragweed given (Fig. 7) or to the pre-treatment hemagglutinin level (Fig. 8). However, all but one of the patients who had reactions after the injection had pre-treatment SSA titers of 500 or more (Fig. 9). Although immunologic studies were performed in 35 cases, 46 were actually treated by ragweed repository injections, 9 of whom were asthmatics. Seven of these 46 patients had severe systemic reactions and 2 had severe local reactions. It is interesting to note that 6 of these marked reactions occurred in asthmatics, none of whom had received treatment in 1959.

DISCUSSION

The objectives of this study were twofold. First, to establish whether the presence or change in concentration of any of the immunologically identifiable constituents present in the serum of an allergic person can be related to the degree of clinical improvement observed after the immunization or "densitization" procedure with aqueous ragweed pollen extract emulsified in oil. Second, to determine whether any correlation exists between the skin sensitizing antibody (SSA), the blocking antibody, and the hemagglutinating antibody, in order to unequivocally ascertain which of the former two factors can be implicated in the agglutination reaction, if indeed either of them can.

It will be apparent from the results obtained that there was no correlation between any of the parameters measured with the exception of one. There was a tendency for reactions to occur following injection when the pre-treatment SSA titer was 500 or higher. It is notable that these results, showing a lack of correlation between the entities measured, are very similar to those described by Arbesman and his co-workers using conventional multiple injection therapy[10, 11] as well as repository therapy.[18]

A number of reports have recently appeared which have implied that the hemagglutinating antibody can be viewed as existing independently from SSA.[9-12] Augustin,[12] using column chromatography, found that hemagglutinating ability could not be separated from blocking antibody activity. Nevertheless, the evidence which has been submitted, either in favor of or against SSA and/or blocking antibody possessing hemagglutinating activity, has been inconclusive.

In order to determine whether SSA could contribute to the increases in hemagglutinin titers observed with some sera after treatment, SSA titers were determined on both pre- and post-treatment sera. It was found, with the exception of 8 cases, that reagin titers did not change appreciably after treatment.

The sera displaying increases in hemagglutinin titers were divided between those which showed marked increases in SSA titers (6 cases) and those in which no significant increase in SSA titers could be detected (12 cases). In view of the fact that hemagglutinating antibody titers increased in sera where SSA concentrations remained constant, we must conclude, as did Arbesman and his group,[11] that reagin and hemagglutinating antibody are distinct entities.

Blocking antibody activity could be detected in only 7 of the 12 sera tested, and of these only 3 showed any increases over pre-treatment values (see Table III). The increase in hemagglutinating activity was not always accompanied by an increase in blocking antibody titer. These results, therefore, as well as those of the SSA experiments, fail to support the theory that hemagglutinating activity should be attributed solely to either SSA or blocking antibody, or to both of these constituents.

Several other interesting conclusions can be drawn from these data. It has been postulated[17] that reagin could be converted to blocking antibody after its reaginic activity is destroyed by heating at 56° C. If this were the case, then one would expect at least a few of the high titer reaginic sera to develop blocking activity after heating. Such was not the case, however. As can be seen in Table III, no blocking antibody activity was detected in the sera of 3 patients, Nos. 6, 10, and 11 (pre- and post-treatment), after heating. They all exhibited high reagin titers. In fact, these titers were higher than in some of the other sera in which blocking antibody activity was manifest after heating. In some of the sera which did possess blocking antibody activity after treatment, no difference could be detected in their pre- and post-treatment reagin titers. One could therefore conclude that reagin is not converted to blocking antibody after heating of the reaginic serum at 56° C. for 1 to 2 hours. This finding also implies that not only does reagin lose its capacity to elicit a triple response but that it loses all ability to react with the ragweed allergen(s).

A second interesting observation concerns the relationship of the hemagglutinating antibody and reagin. As was mentioned above, sera Nos. 6, 10, and 11 (pre- and post-treatment sera; Table III) did not manifest blocking antibody activity after heating. Since the hemagglutinin titers of 2 of these 3 sera were very high, it must also be concluded that the hemagglutinating antibody does not react with the constituent in the ragweed pollen extract to which the skin-sensitizing antibody and blocking antibody are directed. If it did, then it should be able to block the reaction between reagin and the allergen, in the same way as conventional blocking antibody. Since the blocking antibody does react with the allergen in such a way as to inhibit subsequent reaction between the allergen and reagin, it must be directed against the same component of the ragweed pollen extract, if not the same site, as is reagin.

In summary, our interpretation of the results presented above is that hemagglutinating activity is due to a factor different from reagin and blocking antibody. It is certainly not inconceivable that the ragweed pollen extract used in the hyposensitization treatment consists of a heterogeneous mixture of antigens and allergens. It has been demonstrated that ragweed extract (WSR) contains at least eleven well-defined constituents (peptides, proteins, pigments, carbohydrates.[13, 14] Undoubtedly, not all of them are antigenic or allergenic. Moreover, it is unlikely that the only component(s) of WSR to which the affected individual reacts is the allergen. On the contrary, it is quite probable that WSR contains materials which are antigenic, but not allergenic, stimulating the formation of antibody systems unrelated to allergy in the host. The host

would therefore form three varieties of antibodies, a clinically innocuous type of antibody as well as the biologically active antibodies which we designate SSA and blocking antibody. Such a state of affairs exists today insofar as diphtheria toxoid,[15] pneumococcal polysaccharides,[16] and several other common immunizing materials are concerned. In the case of diphtheria toxoid, it has been shown that many of the antibody systems which are known to arise in man can be formed in response to a single complex of antigens.[15]

CONCLUSIONS

1. Sera from 35 patients treated with a single repository injection of ragweed pollen extract were investigated for their skin-sensitizing, blocking, and hemagglutinating titers.

2. There was a definite increase in hemagglutination titer following the desensitization treatment in the majority of patients.

3. There was no correlation between the hemagglutinating titer of any serum and its skin-sensitizing and/or blocking antibody activities.

4. There was no constant relationship between the degree of clinical improvement observed in patients and the concentration of any of the three parameters (hemagglutinin, skin-sensitizing antibody, blocking antibody) measured in the sera of these patients.

5. There was no relation between the clinical response and the quantity of ragweed administered.

6. The incidence of reactions following the repository injection could be correlated with the pre-treatment SSA titer. Most of the severe reactions occurred in asthmatics who had not been treated by hyposensitization procedures in the previous year.

7. Neither the skin-sensitizing antibody nor the blocking antibody contributes appreciably, if at all, to the hemagglutinating capacity of a serum from an allergic individual.

8. It has been demonstrated that reagin is not converted to a blocking antibody upon heating it at 56° C. to destroy its skin sensitizing activity.

9. The results obtained suggest that hemagglutinating antibody does not react with the same constituent(s) of the ragweed pollen extract to which reagin and blocking antibody are directed. These latter two antibodies must be directed to the same constituent of the allergen molecule.

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GROWTH RATE OF CHILDREN WITH INTRACTABLE ASTHMA

Observations on the Influence of the Illness and Its Therapy With Steroids

Constantine J. Falliers, M.D., J. Szentivanyi, M.D., Max McBride, and Samuel C. Bukantz, M.D., Denver, Colo.

MANY of the patients admitted to the Jewish National Home for Asthmatic Children during the past several years showed a marked retardation of growth. The present study was undertaken for the purpose of ascertaining whether the observed growth deficit resulted from hereditary or post-natal influences unrelated to asthma; or whether chronic bronchial asthma and/or its treatment were primarily responsible.

Cohen, Welles and Cohen,[1] in 1940, reported that allergic children were likely to show significant retardation of growth and development, first manifested in loss of weight, then, as symptoms progressed, in suppression of height and skeletal maturity. However, this impairment of growth was not necessarily present in all the cases, and could not be clearly related to the severity of symptoms. In another communication, Cohen and Abram[2] found that the "control of allergy" was followed by "growth repair." Following a personal communication to the authors, Abram transmitted the growth charts of many asthmatic children seen in the pre-steroid era. These charts indicated a considerable variation in growth rates and are under study here.

More recently Van Metre and associates[3, 4] found that asthmatic children who did not receive steroid therapy grew along the normal anthropometric channels. In contrast to this, asthmatic children requiring maintenance anti-inflammatory steroid therapy showed significant reduction in the rate of lineal growth. This reduction was found to be dependent on the type of steroid and related to the average daily maintenance dose per square meter of body surface.

MATERIAL AND METHODS

The subjects of the present review were 302 intractable asthmatic children observed at the Jewish National Home for Asthmatic Children (JNHAC), in Denver, during the 30 month period from July 1, 1958, to Dec. 31, 1960. Of these 302 children, 127 were in residence at the initiation of the study, while the

From the Jewish National Home for Asthmatic Children and Children's Asthma Research Institute and Hospital, 3401 West 19th Ave., Denver 4, Colo.

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remaining 175 were admitted subsequently. The age, on admission, ranged from 5 to 16 years, and the ratio of males to females was almost exactly 2:1, with 200 boys and 102 girls. The length of residence varied from 18 to 24 months with only exceptional patients being discharged after a shorter or longer period of time. During the course of the study, 258 children were observed for 6 months or more, and these only were reviewed in the second part of this investigation concerned with growth in residence.

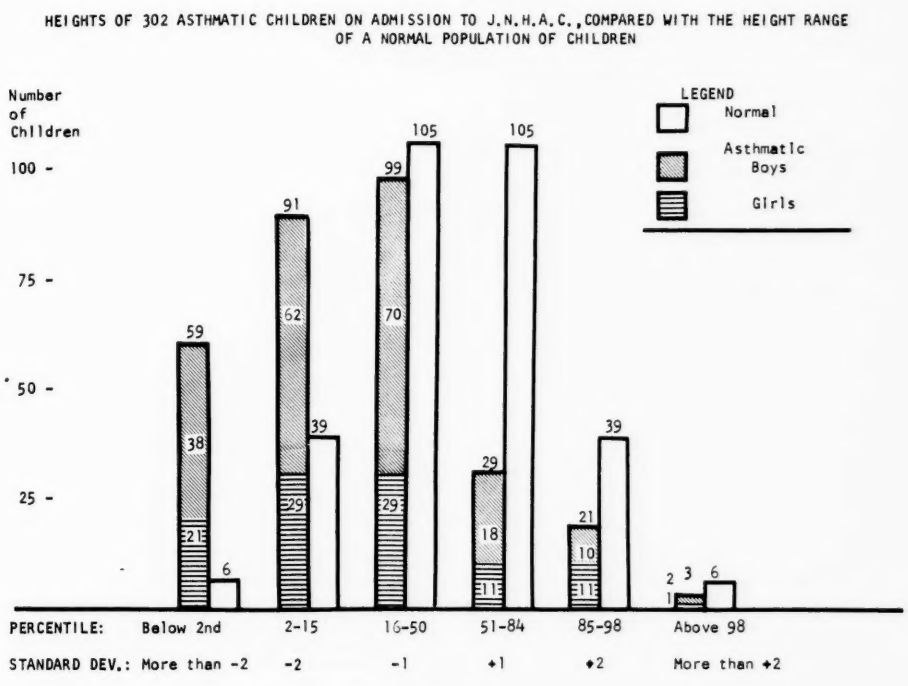

Fig. 1.

Measurements of height and weight were taken on admission and regularly at monthly intervals thereafter. The children receiving anti-inflammatory steroids were seen weekly and, at such times, measurements were obtained. This frequency of measurements was thought more than adequate to obviate errors due to technique. The measurements were plotted on anthropometric charts* and at the same time a note was made in the child's medical record comparing the figures obtained with those of the standard reference tables[11] for a normal population. In order to facilitate grouping and comparison of results with those of others,[12, 15] the position of a subject on the anthropometric scales was expressed in both percentiles and standard deviations from the mean.

Bone age was determined by taking x-rays of the wrist and then by reference to the Radiographic Atlas of Skeletal Development of Greulich and Pyle.[13]

*The anthropometric charts, based on the figures and the design of the Children's Medical Center in Boston and the Iowa Child Welfare Research Station, State University of Iowa, were generously supplied by Mead Johnson and Company, Evansville, Ind.

For comparison of statural growth and osseous development, the degree of deviation of both height and bone age from the mean was expressed in years above or below the child's actual chronological age.

A review of the patients' admission heights indicated a striking deviation toward lower values. Fig. 1 shows the distribution of the admission heights of this population of children as compared to a normal distribution. The biometric data of normal children, illustrated by the empty bars, would be expected to fall along a bell-shaped curve with 50 per cent above and 50 per cent below the mean. It is noted that in 59 of these intractable asthmatics the height was more than two standard deviations below the mean, which is 10 times more than the expected number of 6 for normal children. More than twice the expected number were two standard deviations below, while, on the other side, only 17 per cent were above the mean. Statistical evaluation of these figures revealed a p smaller than 0.0001, which is highly significant.

Review of the admission weights, as shown on Table I, showed a high degree of correlation with heights (correlation coefficient: 0.88 for boys, 0.87 for girls). Only few children, small in stature, showed weights above the mean and these were mostly children on high doses of corticosteroids. Interestingly, there were very few children who were above the mean for height and below the mean for weight. This is not in agreement with previously published studies,[2, 17] where some association was found to exist between asthma and ectomorphism.

TABLE I. CORRELATION OF ADMISSION HEIGHTS AND WEIGHTS

WEIGHT \ HEIGHT	> −2	−2	−1	+1	+2	> +2	TOTALS
> +2	0	1	1	0	0	1	3
+2	1	1	2	6	10	1	21
+1	2	8	16	14	10	9	51
−1	5	25	49	7	0	0	84
−2	12	37	26	4	1	0	80
> −2	39	22	3	0	0	0	63
TOTALS	59	91	99	29	21	3	302

The heights of 117 children were compared with the heights of their parents (expressed in S.D. from the mean), who either accompanied their children on admission or came to visit later. A better correlation was found between the heights of mothers and children (correlation coefficient, 0.7) than between fathers and their children (correlation coefficient, 0.4). The latter figure is slightly lower than those of other studies.[2] The significance of these findings remains to be analyzed.

Following these initial observations, an attempt was made to relate the degree of growth retardation to the age of onset of asthma, the duration of

the illness until admission to the Home, and the pre-admission use of anti-inflammatory steroids. Fig. 2 illustrates graphically the position of each of 200 males in respect to age of onset of asthma, the age at admission, and by subtraction (oblique lines) the duration of the illness. The open circles, squares, and triangles, which are clearly seen to be more numerous, represent children who were one, two, or more standard deviations below the mean, while the black symbols represent those above the mean. Although certain variation among the children regarding the pre-admission severity of asthma could be seen in the admission medical data, as determined by the frequency of hospitalizations,

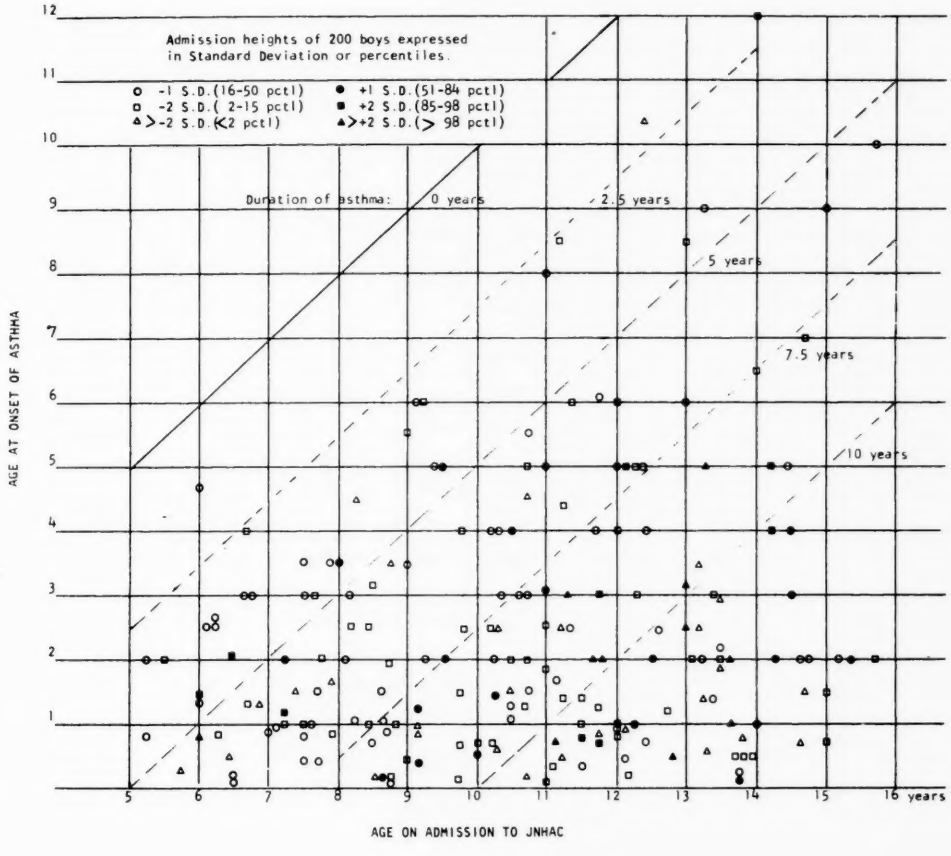

Fig. 2.

the degree of absenteeism from school, and limitations of activity, all these children without exception were known to suffer from chronic perennial and severe asthma, which had not responded to continuous, intensive therapeutic efforts elsewhere and which was the reason for their being referred to and accepted for admission at JNHAC. Fig. 3 illustrates the same data for the 102 girls under study. The information from these charts is summarized in Tables II, A, and II, B. A majority of the children had the onset of their asthma before their third birthday. Statistical evaluation of these data indicated,

however, that there was no significant difference (p 0.5) in the admission heights of children whose asthma started under the age of one, between the ages of one and 3, or after the third year, and that the duration of asthma was not primarily responsible for growth retardation.

Next the records were reviewed to determine the incidence and duration of anti-inflammatory steroid therapy, prior to admission. Unfortunately, even after persistent inquires, it was often impossible to obtain accurate and detailed information as to the exact duration and amounts of steroids employed in the past. Nevertheless, for the purpose of the review, three groups could be easily

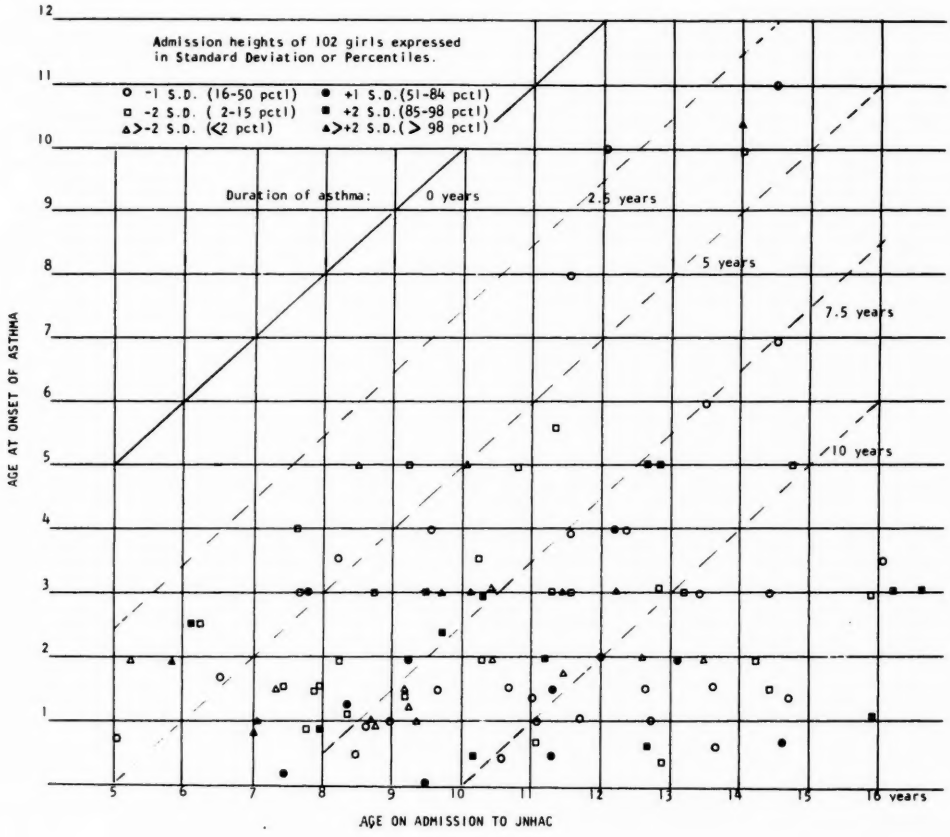
Admission heights of 102 girls expressed in Standard Deviation or Percentiles.
○ -1 S.D. (16-50 pctl)
● +1 S.D.(51-84 pctl)
□ -2 S.D. (2-15 pctl)
■ +2 S.D.(85-98 pctl)
△>-2 S.D. (<2 pctl)
▲>+2 S.D.(> 98 pctl)
Duration of asthma:
0 years
2.5 years
5 years
7.5 years
10 years
AGE AT ONSET OF ASTHMA
AGE ON ADMISSION TO JNHAC
5 6 7 8 9 10 11 12 13 14 15 16 years

Fig. 3.

distinguished (Table II, C): (1) those who had never received anti-inflammatory steroids; (2) those who had received interrupted courses with steroids, ranging from a few weeks to several months per year, and (3) children who had received steroids continuously, or with interruptions no longer than one or 2 weeks, for at least one year prior to admission. In the majority of children in this group, there was a history of steroid administration of several years' duration, sometimes first interrupted and then continuous. There were no children in this group who had been off steroids for any significant period of time during the year preceding admission. Children usually had been given a

TABLE II. HEIGHT DISTRIBUTION OF 302 CHILDREN ON ADMISSION TO JNHAC

	HEIGHT				
	BELOW THE MEAN			ABOVE THE MEAN	
	> −2 S.D.	−2 S.D.	−1 S.D.	+1, 2 OR MORE S.D.	TOTALS
A. In Relation to the Age of Onset of Asthma					
AGE					
Under 1 year	20	25	26	16	87
1-3 years	28	41	43	28	140
Over 3 years	11	25	30	9	75
Totals	59	91	99	53	302
B. In Relation to the Duration of Asthma					
DURATION					
Less than 5 years	5	11	23	8	47
5-10 years	37	55	52	26	170
More than 10 years	17	25	24	19	85
Totals	59	91	99	53	302
C. In Relation to Pre-admission Steroid Therapy					
STEROID THERAPY					
None	4	14	23	8	49
Interrupted*	24	49	51	34	158
Continuous†	31	28	25	11	95
Totals	59	91	99	53	302

*One to 50 per cent of one year.
†For at least one year prior to admission.

variety of anti-inflammatory steroids, often reflecting the new developments in the pharmaceutical industry. In recent years, the most commonly employed steroids, prior to the child's admission to the Home, were prednisone, prednisolone, methylprednisolone, triamcinolone, and dexamethasone. A positive trend but no definite correlation was noted between early onset of asthma and the introduction and maintenance of anti-inflammatory steroid therapy, with p smaller than 0.1.

In comparing the figures on the extreme left column of Table II, C, one can notice that 8 per cent (4 of 49) of the children (4 times the normal number) who had not received steroids in the past were more than two standard deviations below the mean in height at admission. Compared to this, 15 per cent (24 of 158) of the children who had received intermittent steroids were in this category, and 32.5 per cent (31 of 95) of the children treated with continuous steroids belonged to this very retarded group. In the second column containing those figures within two standard deviations below the mean, no significant difference is noted, whereas in the categories nearer and above the mean the children who had received continuous steroid therapy were represented in smaller numbers. It is noteworthy that, among the children whose height was above the mean on admission, those who had received intermittent steroid therapy are represented in larger numbers (22 per cent). These differences shown on Table II, C, were found to be statistically significant, with a p smaller than 0.01.

In the second part of this study, concerned with the course in residence, the physical growth of these children was evaluated under the close observation which is possible in a residential treatment center.[6]

Within the first 6 months after admission, the children at the Home could be classified into four distinct categories:

1. The first group comprises those children who lost all asthmatic symptoms upon admission, were taken off all medication, and remained asthma-free during their entire residence. Rarely, once or twice a year, these children did have a wheezing spell, and invariably this episode responded promptly to the administration of an oral or inhaled bronchodilator drug.

2. The second category consisted of children who, although not as ill as their pre-admission history indicated, yet continued to wheeze most of the time and had frequent episodes of asthma, usually several times a month. These children could be well controlled with oral or inhaled bronchodilator drugs, and occasionally also needed subcutaneous injection of epinephrine or rectal instillation of aminophylline. Their activity was, as a rule, not restricted.

3. The third category is the least homogenous one. They were children like those in the second category, except that at times they developed acute, severe attacks of asthma, not controllable without steroids. The frequency and duration of the steroid administration varied considerably.

4. It was noted that anyone who required steroids more than 50 per cent of the period of observation practically was on steroids all the time. Therefore, the fourth category is considered quite a distinct one. These were the children who, as a rule, had been on anti-inflammatory steroids for several years prior to admission, and these drugs could not be withdrawn after the children were admitted to JNHAC. Repeated attempts to discontinue the drug or reduce it below a critical level, quite well defined for each child, resulted in severe attacks of asthma, often status asthmaticus, exacerbations which could not be controlled without the resumption of higher doses of steroids.

The 258 children, 172 boys and 86 girls, who had been under observation for 6 months or more by Dec. 31, 1960, were the subjects of this part of the study. Table III shows the number of children in each of the four categories listed above, as well as the number of measurements used in calculating the means. Lineal growth was expressed in semi-annual increments in centimeters and compared with the data for normals available from the Child Research Council of the University of Colorado.[15] For each age group, from 5 to 16, the mean increments were calculated from the individual measurements obtained. Fig. 4 shows these mean values of the semi-annual increments of 172 males distinguishing categories 1, 2, 3, and 4 described above and comparing them to the normal means. A clearly defined suppression of growth is seen for the category of asthmatic children on continuous anti-inflammatory steroid therapy and this seems to be true for the entire age range covered. In contrast, the subjects in categories 1, 2, and 3 were found to grow at rates within the normal range. Fig. 5 contains the same information for 86 girls. The same four groups are compared to the normal means, which, as illustrated, are somewhat different from the boys', showing an earlier adolescent spurt. The growth lines, obtained by connecting the means for each age, appear more irregular

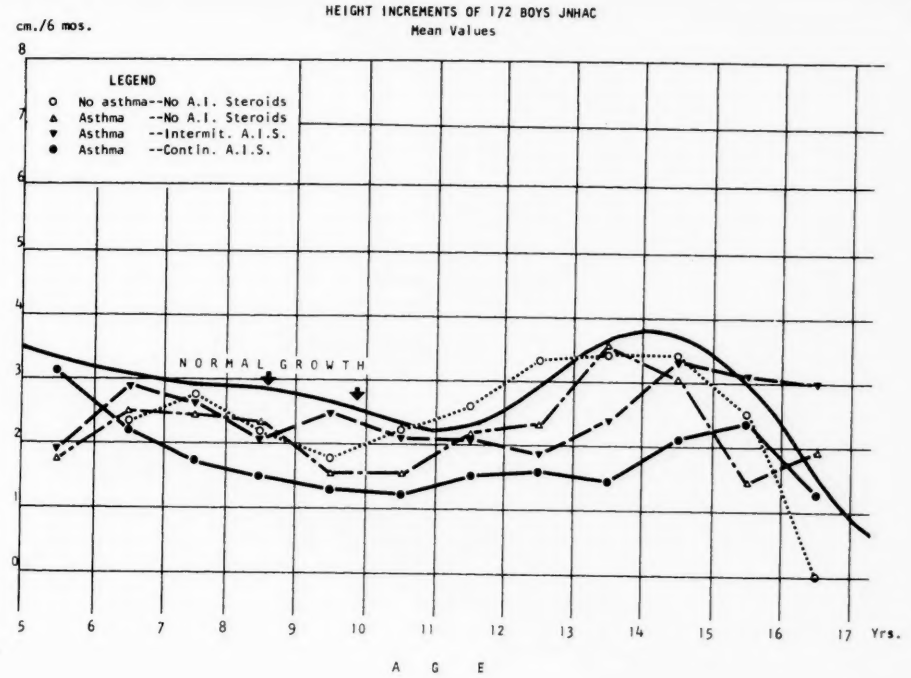
HEIGHT INCREMENTS OF 172 BOYS JNHAC
Mean Values
cm./6 mos.
LEGEND
No asthma--No A.I. Steroids
Asthma --No A.I. Steroids
Asthma --Intermit. A.I.S.
Asthma --Contin. A.I.S.
NORMAL GROWTH
0 1 2 3 4 5 6 7 8
5 6 7 8 9 10 11 12 13 14 15 16 17 Yrs.
A G E

Fig. 4.

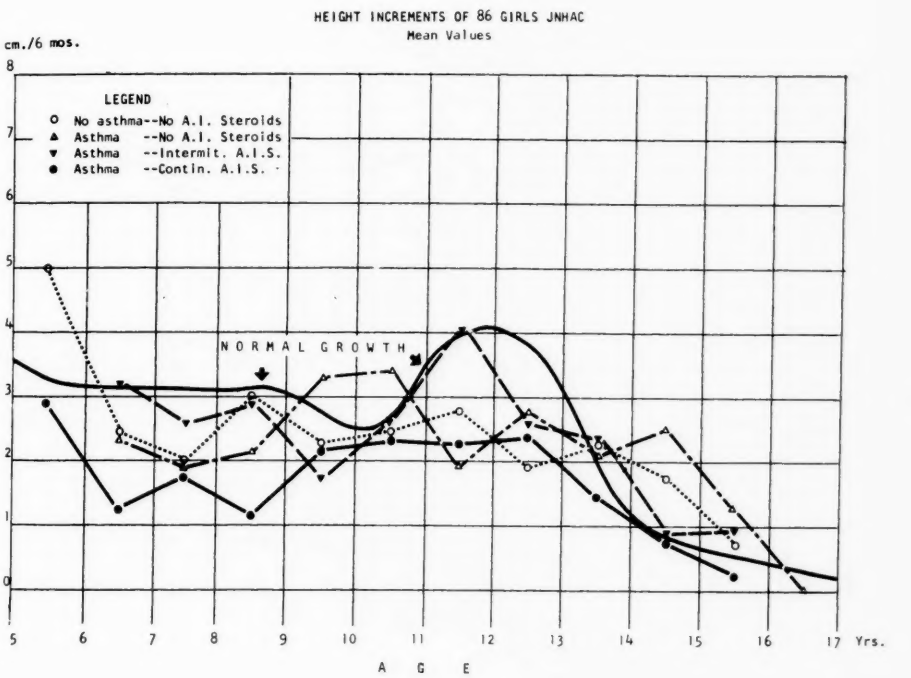
HEIGHT INCREMENTS OF 86 GIRLS JNHAC
Mean Values
cm./6 mos.
LEGEND
No asthma--No A.I. Steroids
Asthma --No A.I. Steroids
Asthma --Intermit. A.I.S.
Asthma --Contin. A.I.S.
NORMAL GROWTH
0 1 2 3 4 5 6 7 8
5 6 7 8 9 10 11 12 13 14 15 16 17 Yrs.
A G E

Fig. 5.

than those for boys, and this may be partly due to the smaller number of girls studied, which permitted individual variations to influence the means in opposite directions.

TABLE III. OBSERVATIONS OF GROWTH IN RESIDENCE AT JNHAC

CATEGORY	BOYS		GIRLS	
	NUMBER OF CASES	NUMBER OF SEMI-ANNUAL MEASUREMENTS	NUMBER OF CASES	NUMBER OF SEMI-ANNUAL MEASUREMENTS
No asthma; no medication	64	159	35	104
Asthma; treated symptomatically, no steroids	40	106	15	35
Asthma; treated symptomatically, including courses of steroids*	29	86	14	41
Asthma and continuous steroid therapy†	39	106	22	70
Totals	172	457	86	160

*Not exceeding 50 per cent of the observation period.
†No interruption longer than one week for any 6 month period.

Further analysis of all individuals on continuous anti-inflammatory steroids revealed a wide scatter of values, ranging from zero to above the normal mean for any particular age, and more marked among adolescents. In view of this fact, and also of the reported observation[4] that the average daily dose of steroid is a critical factor determining growth arrest, the records of 31 children admitted to the Home after July 1, 1958, who had required continuous therapy with steroids, were reviewed in detail. Children in whom steroid therapy was interrupted for not more than one week, during the observation period, are also included in this category. A careful record is kept of all medication prescribed and taken during a child's residence at the Home, and therefore precise and reliable information regarding the exact amount of steroid taken by a child is readily available. The total amount of the steroid, as well as the minimum and maximum daily doses, were recorded for each month and the sums calculated for any observation period. Each child's total body surface area, in square meters, was obtained from the nomogram of Dubois on the basis of the weights and heights, at the beginning and end of the observation period. Then the average daily dose of the steroid per square meter of body surface was calculated.

Thirty of these "steroid-dependent"[22] children were on prednisone and one on methylprednisolone at the beginning of the observation period and for a length of time indicated on Table IV. During the last 12 months of the study, 12 of the children on prednisone and one on methylprednisolone were changed to an equivalent amount of cortisone in an effort to ascertain the relative anti-inflammatory and growth suppressing effect of cortisone in comparison to the other analogues. Twenty-five milligrams of cortisone was considered equivalent to 5 mg. of prednisone, or 4 mg. of methylprednisolone.

Table IV lists these 31 children, their sex and age, the length of the observation period, the average daily dose of steroid, as well as the maximum and minimum amounts given per day. The growth rate is expressed as per cent of

normal, taking for "normal" the mean semi-annual increment rates observed at the Child Research Council of the University of Colorado. Of the 31 children on prednisone, 12 grew at the rate of 70 to 130 per cent of normal, or a mean of 91 per cent of the normal. The mean daily prednisone intake for this group of 12 children was 5.9 mg. per square meter of body surface. In contrast to this, the average daily dose of prednisone for 21 children, during periods when they grew less than 50 per cent of the normal, was 9.5 mg. per square meter.

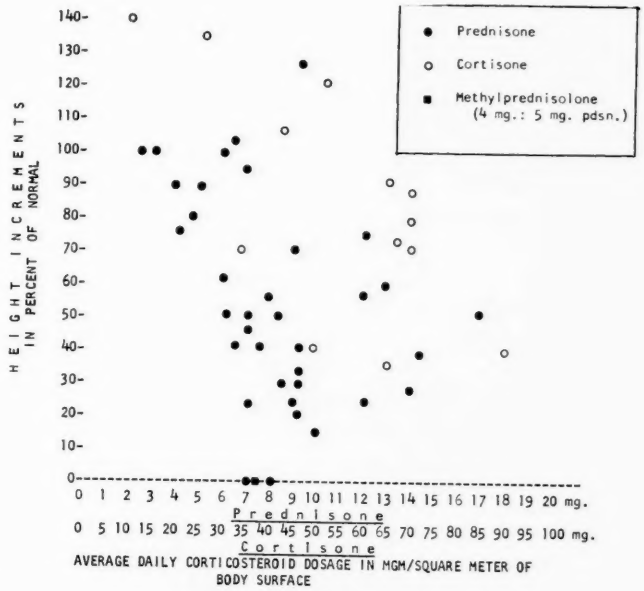

Fig. 6.

All patients who received cortisone were previously on either prednisone or methylprednisolone. In 3 of them (D.B., D.C., M.I.) no noticeable acceleration of growth was noted. Of them, D.B. was a tall 14-year-old boy, who could well have been in the phase of post-adolescent growth deceleration. The remaining 10 children grew remarkably better when placed on cortisone, and some of them experienced an acceleration of linear growth exceeding the normal for their age. These 10 children, before they were placed on cortisone, grew at an average rate of 37 per cent of the normal, and were on an average daily maintenance dose of 9.7 mg. per square meter per day of prednisone. After they were placed on cortisone, the average growth, for this group of 10, was 97 per cent of normal, with a range from 70 to 140 per cent. The average daily maintenance dose of cortisone was 50.5 mg. per square meter. Fig. 6 graphically illustrates the results of our observations. It can be seen that children on cortisone (open circles), in general, grew better than those on prednisone or methylprednisolone. It is also indicated in this figure that several of these children required more cortisone than the accepted ratio of 5:1 with prednisone would have led us to believe. In our experience, the children needed an average of

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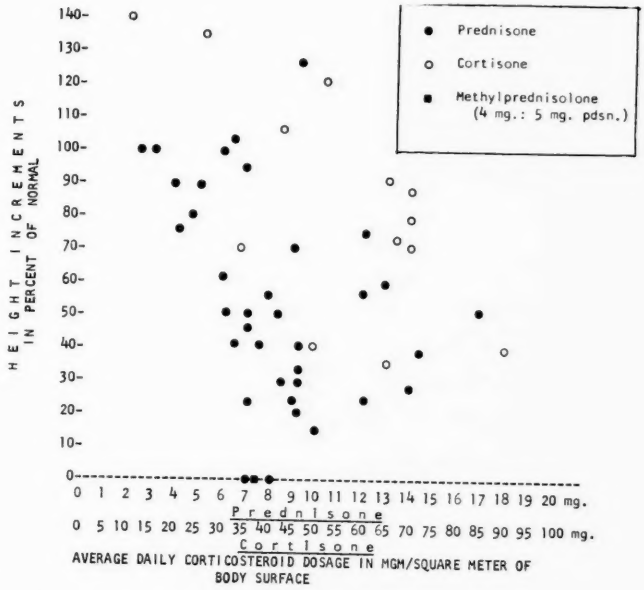

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TABLE IV. HEIGHT INCREMENTS OF 31 CHILDREN ON MAINTENANCE ANTI-INFLAMMATORY STEROID THERAPY

			PREDNISONE				CORTISONE			
PATIENT	SEX	AGE ON ADMISSION	DURATION (MONTHS)	DOSE RANGE	AVG. DOSE (MG./M.²/ DAY)	LINEAR GROWTH (% OF NORMAL)	DURATION (MONTHS)	DOSE RANGE	AVG. DOSE (MG./M.²/ DAY)	LINEAR GROWTH (% OF NORMAL)
DB	M	14	8	7.5-30	8.7	27	10	75-150	65	35
DB	F	11½	7	0-30	7	98				
MB	F	11½	6	5-20	7.5	0				
			15	2.5-25	4	75				
MB	M	13	9	10-40	14	25	2	100-150	71	70
PC	M	8½	10*	2-16	7.2	0	11	25-150	43	105
DC	M	8	6	2.5-40	9	40				
			8	5-30	5.9	60				
JSC	F	8	16	0-30	7	0				
			7	2.5-15	5	80				
EC	F	8½	7	2.5-30	8	65				
JD	M	12	8	2.5-40	7	50				
JD	M	10	23	2.5-30	6.5	40				
ME	M	12	12	2.5-10	2.5	95				
RG	M	11	12	2.5-40	12	55				
AG	M	10	23	2.5-30	7	50				
MI	M	6½	14	5-40	18	48	12	37.5-300	90	40
SK	F	11	14	2.5-30	5	90				
JL	M	13	7	10-40	15	42				
MM	F	10	9	2.5-40	13	70				
DM	F	8½	9	2.5-30	10	15	11	37.5-150	55	120
BM	M	10	15	2.5-15	3.5	95				
DM	M	14½	6	2.5-40	8.7	63				
WM	M	8	6	2.5-40	6.5	100				
DN	F	8½	11	2.5-30	8.3	30				
			12	5-30	14.5	36				
RP	F	8½	6	5-30	12	75	7	25-200	70	80
			11	5-30	9	20				
RP	M	9	7	2.5-20	4	85	3	12.5-100	10	140
EP	M	14½	6	5-20	9	37	8	25-200	68	70
SP	M	9½	12	2.5-30	6	45				
RR	M	11½	3	5-15	8.1	50	8	20-100	27	135
RT	M	7	12	2.5-40	9	30				
			13	2.5-30	6	100				
SW	F	9½	4	2.5-30	12	23	6	25-200	65	90
AW	F	14	14	7.5-30	7	33	2	37.5-150	33	70
JZ	M	16	16	2.5-45	9.5	130				

*Methylprednisolone.

6.5 times more cortisone than prednisone (in milligrams) for adequate anti-asthmatic effect. Indeed, generally, control of asthma with cortisone was not as satisfactory as with prednisone, and perhaps even larger doses of cortisone should have been used. Although, for several patients on cortisone, salt restriction and additional potassium chloride were prescribed, the weight gain and "Cushingoid" appearance were more marked with cortisone than with the other analogues. A brief trial of dexamethasone, from November, 1958, to April, 1959,[7] indicated that the use of this steroid, in comparable doses, is accompanied by even more marked weight gain and "Cushingoid" features than either prednisone or cortisone. The short duration of this trial did not permit an evaluation of its effect upon linear growth.

Through the courtesy of Dr. W. H. Daughaday of Washington University of St. Louis, determination of the serum sulfation factor activity[10] was performed on 6 children as a means of evaluating the growth hormone activity of the anterior pituitary gland. As shown on Table V, 3 of these children were on anti-inflammatory steroid therapy and 3 were not. Some had severe asthma, others did not. Growth rate varied considerably, from 30 to 140 per cent of normal. Only one subject, E.P., a 14-year-old boy with chronic, severe asthma and advanced bilateral bronchiectasis who had been on perdnisone for over one year and cortisone for 6 months prior to the assay, showed activity below the normal range. These results appeared to warrant additional studies.

TABLE V. SULFATION FACTOR ACTIVITY OF SERUM IN ASTHMATIC CHILDREN IN RELATION TO LINEAR GROWTH

PATIENT	SEX	AGE	HEIGHT (INCHES)	PERCENTILE	LINEAR GROWTH RATE (PER CENT OF NORMAL)	AVERAGE DAILY C-STEROID (MG./M.^{2})	ASTHMA	SULFATION FACTOR ACTIVITY
J. Z.	M	18	63	10th	125	Prednisone 9.5 mg.	Severe	0.498 unit*
W. F.	M	8	45	below 3rd	102	Prednisone 6 mg.	Severe	1.19
C. L.	M	14	68	90th	110	None	None	0.649
J. C.	M	12½	52½	below 3rd	65	None	Minimal	0.720
R. L.	M	15	61	3rd	75	None for 15 mo.	None for 6 mo.	2.22
E. P.	M	15	54½	below 3rd	45	Cortisone 68 mg.	Severe, also advanced bronchiectasis	0.034

*Normal range 0.5-1.5 units, in reference to standard serum.

COMMENT

Although measurement of height is among the simplest biometric procedures, the evaluation of the growth rate of an individual, or a group of individuals, is still a very complex problem.[15, 16]

There are first problems of technique,[12, 15] such as obtaining measurements under identical environmental conditions, at the same time of the day, and, if possible, by the same individual. Variability in these factors was found to result in significant modifications of results.[15]

Once adequate data have been compiled, the problem of assigning some meaning to them arises. Should increments be expressed in absolute numbers, per unit of time, or as increments in per cent of the total? And how should the growth rate of a smaller individual be compared with that of a larger one, of the same age and sex, or of different sex or different age?

Investigators in this field have available the established normal data compiled by previous workers during the past decades.[11, 12, 15] Nevertheless, the question whether one population can rightfully be compared with another, from which the so-called "normal values" are derived, remains an open one.

The problem becomes even more complex when one attempts to study growth of a population of children suffering from a prolonged illness[9, 16] and receiving a variety of diets and medicaments. In the case of perennial and severe bronchial asthma, the multiplicity of factors to be considered is immense. The degree of incapacitation the illness imposes on the child, which often seriously limits his activity and reduces his appetite, may logically be considered a factor influencing growth.[11] Then comes the question of respiratory insufficiency with hypoxia, as it is well known that conditions interfering with adequate supply of oxygen to the periphery will inhibit growth.[20] In addition, the asthmatic child, especially the chronic case, will, almost without exception, be subjected to dietary trials which often eliminate important nutrients from his diet.[11, 12] There is also the question of heredity, as a certain body type has been linked with higher incidence of asthma.[2, 17] And last, but not least, comes the problem of drug therapy. The administration of such common anti-asthmatic drugs as iodide salts or theophylline, which often are administered continuously for months or years, may result in nausea or gastric irritation and loss of appetite, with consequent suboptimal food intake. In a small number of cases, iodides may possibly affect growth by suppressing thyroid function.[19]

The prolonged administration of the anti-inflammatory adrenal-cortical steroids is considered a major factor in inhibiting growth.[3, 4, 5, 9] Various cortisone analogues were found to differ in their degree of anti-inflammatory and growth inhibiting effects.[3, 4]

The present study demonstrated that, although on admission the entire population of asthmatic children showed a trend toward lower values in stature, children who had been continuously on adrenal steroids were more likely to be markedly retarded in growth. Observations during the child's residence at the Home also indicated that prolonged administration of steroids suppressed growth rates, while children with asthma, not on steroids or those receiving only interrupted courses, grew as well as those who remained free of asthma. The question remains unanswered whether children with severe asthma requiring continuous maintenance therapy with anti-inflammatory steroids would have grown any better had they not been on steroids. Individual observations, so far, indicate that severe asthma alone may equally suppress growth.

One of the main points, the authors wish to stress, is that of individual variation in anthropometric measurements. This variation often was so overwhelming as to discourage any attempts at calculation of means and statistical

evaluation. In this era, when only statistically validated results are scientifically acceptable, the individual tends to become lost.[18] Quite frequently, however, all the tables and growth curves cannot help one predict the behavior of an individual patient regarding growth expectancy. Certain children, and this was seen in all categories of patients, including those continuously on steroids, have shown entirely normal growth patterns and many exhibited perfect adolescent spurts, while others have lagged significantly behind. It is, therefore, with the greatest reservations that we present these preliminary findings with the hope that the individual will not be forgotten, and that the general impressions derived from our computations will serve as another step for further investigations.

SUMMARY

Anthropometric data were collected on 302 intractable asthmatic children, 6 to 16 years of age, observed at the Jewish National Home for Asthmatic Children in Denver, from July 1, 1958, to Dec. 31, 1960.

Admission heights of 83 per cent of these children fell below the mean, or the fiftieth percentile, for their respective age and sex and, in 20 per cent or 10 times more than normal, the heights were more than two standard deviations below the mean.

Weights generally corresponded with heights. Bone age, paralleled the heights rather than the chronological age, although a fairly wide range of values was noted. The size of parents did not seem to be primarily responsible for the observed growth deficit among asthmatic children.

A review of the individual pre-admission histories indicated that an earlier onset of asthma or a longer duration of the illness was not associated with a significantly greater degree of growth retardation. A history of continuous administration of anti-inflammatory steroids before admission was more common among the smaller children, while shorter courses of steroids, not exceeding 50 per cent of any year of life, did not seem to affect growth adversely.

During the 18 to 24 months of residence at the Home, the presence of asthmatic symptoms did not diminish height increments, neither did brief courses of steroids affect growth. Continuous maintenance steroid therapy, given only in severe cases of asthma uncontrollable by other means, was associated with a significantly reduced linear growth rate.

A comparison of the growth suppressing effect of prednisone and cortisone indicated that children requiring mean daily doses of prednisone, exceeding 6 mg. per square meter of body surface, exhibited reduced lineal growth rates. On the contrary, children receiving cortisone, in mean daily doses exceeding 50 mg. per square meter, exhibited normal growth increments. The control of asthma, however, seemed to be less satisfactory with cortisone than with prednisone, in what are considered equivalent amounts.

A considerable individual variation was noted in growth rates among children in all categories and especially among the adolescents. Preliminary

results of pituitary function assays, by means of serum sulfation factor activities, are reported, and the need for additional studies is emphasized.

Acknowledgment is made of the very helpful suggestions given by Drs. E. Boyd, M. M. Maresh, and C. F. Hansman of the Child Research Council, University of Colorado School of Medicine. The statistical studies were carried out by Kenneth Purcell, Ph.D., and Luis Tan, M.D., and the radiological estimation of bone age was done with the assistance of Jacqueline Jorgensen, M.D., of the JNHAC staff.

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IMMUNOLOGIC STUDIES OF REPOSITORY RAGWEED POLLEN THERAPY

Carl E. Arbesman, M.D., and Robert E. Reisman, M.D., Buffalo, N. Y.*

It has been shown that after multiple injection therapy with pollen extracts, a thermostabile blocking antibody is produced.[1] There is usually no change in the skin-sensitizing antibody titer during hyposensitization therapy, but one investigator[2] reported a temporary early increase in this antibody response. More recently, we[3] and others[4, 5] have shown that there is an increase in the hemagglutinating antibody titer following multiple aqueous pollen injection therapy as measured by either the tanned cell or bis-diazotized benzidine technique. Unfortunately, neither the significance of this antigenic response nor the apparent lack of correlation between the change in blocking, skin-sensitizing, or hemagglutinating antibodies to each other, or to the clinical response of the patients is, as yet, explained.

Repository pollen therapy has been reported by several investigators to be at least as effective in the relief of pollinosis as the conventional multiple injection therapy. Nevertheless, there has been a paucity of information on the immunologic response following pollen emulsion therapy. However, Loveless[6] noted that blocking antibody titers were increased and sustained by repository therapy.

In view of these facts, it was decided to critically study this phase by means of determining the change in blocking, reagin, and hemagglutination antibody titers in patients who received either the repository pollen, a placebo emulsion, or the usual multiple aqueous injection therapy.

METHODS AND PROCEDURES

1. *Source and Preparation of Emulsions.*—

As a member of the Committee on Specific Therapy of the American Academy of Allergy, we received our material from the Chairman, Dr. Samuel Feinberg. One part of defatted ragweed pollen extract was added to two parts of 10 per cent Arlacel A in Drakeol VR6 and placed in sterile vials with neo-

From the Departments of Medicine and of Bacteriology and Immunology, University of Buffalo School of Medicine, and the Allergy Research Laboratory and Allergy Clinics of the Buffalo General Hospital and Children's Hospital.

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prene stoppers. The final concentrations of the ragweed pollen extract in this mixture available to the investigator varied from 500 to 20,000 pollen units. These mixtures were emulsified for 30 minutes by a Conscot* apparatus several days prior to use, and again for 10 minutes just prior to injection into patients. The emulsions were all examined microscopically, by centrifugation, and by scratch tests in sensitive individuals. The emulsions were transferred to 2.0 c.c. disposable syringes and 1.0 c.c. was injected at one time, in one site, deep subcutaneously.

2. *Selection of Patients.*—

Ragweed-sensitive patients from the Allergy Clinics of the Buffalo General and Children's Hospitals and from private practice were used in this study and were divided into two separate investigations.

A. *Double-blind study:* Patients who had been on perennial ragweed therapy were divided, at random, into three groups by one of the investigators. The groupings were unknown to the other participants† in this study who evaluated the clinical results. One group of patients received 20,000 pollen units in repository form, another group was given only a saline oil emulsion without the pollen extract, and the third group was continued on its regular aqueous perennial therapy.

B. *Pre-seasonal study:* Ragweed-sensitive patients who never had previous specific therapy, or had no therapy in 1960, were given from three to eight primer doses of aqueous extracts prior to the single injection of emulsion. The amount of pollen in the emulsion was between 25 to 50 times the concentration of the last aqueous dose. This was considered to be a safely tolerable dose on the basis of a maximum of 2 per cent free antigen (not in emulsion phase) present in a "good" emulsion. The repository therapy was given at varying intervals from 3 to 11 weeks prior to the onset of the ragweed pollen season.

Samples of sera were obtained from each patient prior to (1) aqueous primer doses, (2) the repository injection, (3) the ragweed pollen season, and (4) after the ragweed pollen season. These sera were frozen until all samples were collected and ready for immunologic investigation.

Sera from the patients in the double-blind study were taken prior to the repository injection, prior to the pollen season, and again at the end of the season.

3. *Immunologic Methods.*—

A. *Hemagglutination titers* of all samples of the sera collected were determined by the tanned cell technique as previously described.[7] All samples from an individual patient were tested at the same time. The reciprocal of the highest dilution of serum which still caused an agglutination of tanned red blood cells coated with ragweed pollen extract was called the titer.

B. *Reagin antibody titers:* The studies on change in reaginic and blocking antibody titers were limited to 14 patients of the pre-seasonal group. All sera

*Manufactured by Conscot Company, Rockaway, New Jersey.

†The attending physicians in the Allergy Clinics, who evaluated the clinical response, were Dr. Charles Banas, Dr. Thelma Brock, Dr. Doris Rapp, and Dr. Leonard Wolin.

were cultured for sterility and each of the donors (patients) were carefully questioned as to a previous history of jaundice or hepatitis.

Serial "five"-fold dilutions of the various serum samples were injected intradermally in 2 nonatopic recipients. Twenty-four to 48 hours later each site was tested with a constant amount of ragweed pollen extract. The reciprocal of the highest dilution of serum which positively sensitized the skin was considered the titer of the serum.

C. *Blocking antibody titers:* Blocking antibody titers were determined, in each of 2 recipients, according to the classical method of in vitro neutralization, as described by Loveless.[1]

D. *Scratch test titrations:* Serial dilutions of ragweed pollen extract were used for scratch tests on the patients who received the repository treatment and 13 who received placebo injections. The highest dilution of pollen extract which still gave a positive reaction was determined prior to repository therapy, prior to the pollen season, and after the pollen season.

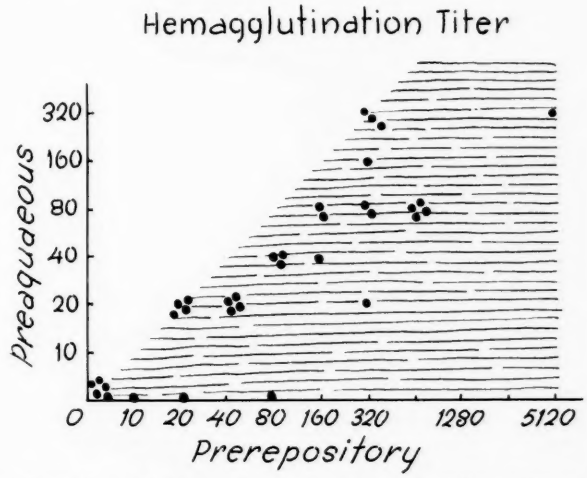

Fig. 1.—The effect of small doses of aqueous ragweed pollen extract on the tanned cell hemagglutination titer prior to the injection of the repository emulsion. Each dot represents the comparative titers. All symbols in the shaded area represent an increased titer.

RESULTS: IMMUNOLOGIC RESPONSE

A. *Hemagglutination.*—

1. *Pre-aqueous to pre-repository:* Of 38 patients from whom we obtained serum samples prior to their aqueous therapy (3 to 8 injections) and before the repository injection, 23 (60 per cent) showed an increase in titer even with this minimal dosage of pollen extract injected. Twelve of these 23 patients (34 per cent) had a fourfold increase or greater in titer (Fig. 1; Table I).

TABLE I. HEMAGGLUTINATION TITERS (PRE-AQUEOUS TO PRE-REPOSITORY)

NUMBER OF PATIENTS	INCREASE IN TITER		
	2-FOLD	4-FOLD	> 4-FOLD
38	11	4	8

2. *Pre-repository to pre-season:* Comparison of the hemagglutination titers just prior to the injection of emulsion and before the season is graphically demonstrated in Fig. 2. Twenty-nine of the 61 patients in the pre-seasonal group had an increase in hemagglutination titer. The titer remained unchanged in 18, 11 showed a twofold decrease, and 3 showed a fourfold or greater decrease (Table II).

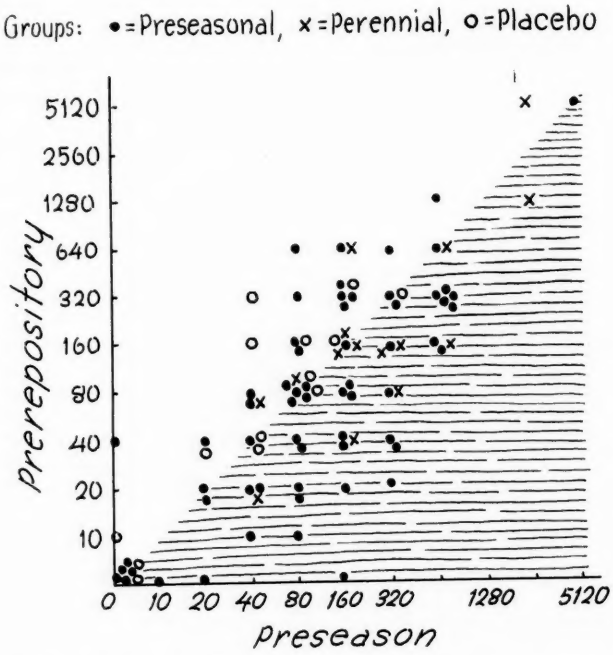

Fig. 2.—Change in tanned cell hemagglutination titer after repository pollen therapy and prior to the onset of the ragweed pollen season. Symbols in shaded area demonstrate an increase in titer.

TABLE II. HEMAGGLUTINATION TITERS (PRE-REPOSITORY TO PRE-SEASON)

GROUP	NUMBER OF PATIENTS	INCREASE			SAME	DECREASE		
		2-FOLD	4-FOLD	> 4-FOLD		2-FOLD	4-FOLD	> 4-FOLD
Pre-season	61	14	9	6	18	11	2	1
Perennial	15	4	3		5	2	1	
Placebo	14	0			8	4	2	

Of the 15 patients who had been on perennial therapy and were then given a pollen emulsion injection, the titer increased in 7, was unchanged in 5, and decreased in 3.

The hemagglutination titer of those patients previously treated with perennial ragweed therapy who received the oil emulsion without pollen remained unchanged in 8 and decreased in 6. There was no increase of titer in this group of patients (Fig. 2).

3. *Pre-season to post-season:* In order to determine any effect on the hemagglutination titer by either the inhalation of pollen during the season or

any further delayed antigenic response from the repository injection, the titers just prior to the pollen season and following the disappearance of ragweed from the air were compared.

Of the 62 patients in the pre-season group, the titers remained the same in 26, decreased in 31, and increased in only 5 (Table III). Of the perennial group, 10 of 14 had a decrease, and of the placebo group, 10 of 16 had the same titer (Fig. 3).

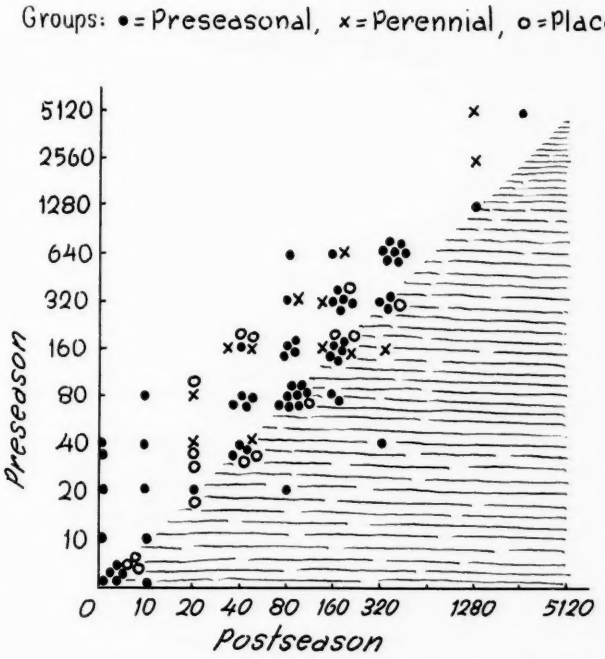

Fig. 3.—Change in tanned cell hemagglutination titer prior to and after the ragweed pollen season. The symbols in the shaded area indicate an increase in titer.

TABLE III. HEMAGGLUTININ TITERS (PRE-SEASON TO POST-SEASON)

GROUP	NUMBER OF PATIENTS	INCREASE			SAME	DECREASE		
		2-FOLD	4-FOLD	> 4-FOLD		2-FOLD	4-FOLD	> 4-FOLD
Pre-season	62	3	2		26	31		
Perennial	14	1			3	4	6	
Placebo	16	0			10	3	3	

4. *Pre-repository to post-season:* The changes in hemagglutination titer of the sera taken just prior to the repository injection and following the ragweed pollen season are illustrated in Fig. 4 and tabulated in Table IV. Of the 61 patients in the pre-seasonal group, 11 had a twofold increase in titer and 6 had a fourfold or greater increase. The titers in 23 patients remained unchanged, but 21 showed a decrease in titer. Of the perennial and placebo groups, there was not one patient who had a fourfold increase in hemagglutination titer. However, 8 of the 14 patients who had stopped perennial aqueous therapy and had a placebo injection had a decrease in titer.

TABLE IV. HEMAGGLUTINATION TITERS (PRE-REPOSITORY TO POST-SEASON)

GROUP	NUMBER OF PATIENTS	INCREASE			SAME	DECREASE		
		2-FOLD	4-FOLD	> 4-FOLD		2-FOLD	4-FOLD	> 4-FOLD
Pre-season	61	11	3	3	23	13	5	3
Perennial	15	2			9	2	2	
Placebo	14	1			5	3	2	3

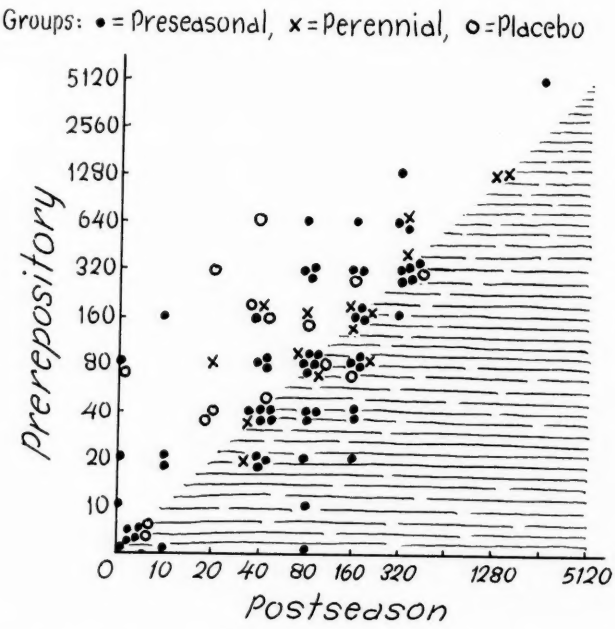

Fig. 4.—Comparative tanned cell hemagglutination titers prior to repository therapy and after the ragweed pollen season. The symbols in the shaded area indicate an increase in titer.

5. *Clinical response and hemagglutination titers:* Thirty-five patients in the pre-seasonal repository group had a satisfactory clinical response. Of these, only 6 had a fourfold or greater increase in hemagglutination titer (pre-repository to pre-season). Seven of the remaining 28 patients who did not benefit from repository treatment likewise had an increase in hemagglutination titer (Table V).

TABLE V

CLINICAL RESPONSE	NUMBER OF PATIENTS	CHANGE IN HEMAGGLUTINATION TITER*		
		INCREASE†	SAME	DECREASE
Not improved	35	6	28	1
Improved‡	28	7	20	1

*Pre-repository to pre-season.
†At least a fourfold increase.
‡At least 50 per cent benefit.

These data confirm our previously reported studies that the hemagglutination response had no relationship to the clinical response.

B. *Reagin (Skin-Sensitizing Antibody).*—

1. The skin-sensitizing antibody titers of the 14 patients of the pre-season group treated with repository ragweed extract were determined in each of 2 recipients. These patients were arbitrarily chosen on the basis of repository dosage, so as to represent the range of 500 to 10,000 pollen units. As can be seen in Table VI, 4 patients showed an increase in reagin titer, 3 a decrease, and 7 remained unchanged. There was no relationship between dosage of repository given and change in skin-sensitizing antibody titer.

TABLE VI. REPOSITORY DOSAGE TO REAGIN ANTIBODY RESPONSE

DOSAGE (POLLEN UNITS)	NUMBER OF PATIENTS	INCREASE	DECREASE	SAME
500	3	1		2
1,000	3		1	2
2,500	3	2		1
5,000	2	1	1	
10,000	3		1	2
Totals	14	4	3	7

2. There was, likewise, no relationship between the clinical response and change in reagin titer (Table VII). Of the 9 patients who were clinically improved, 3 demonstrated an increase in skin-sensitizing antibody. One of the 5 patients who did not improve, likewise, showed an increase in reaginic antibody. The inhalation of pollen during the ragweed season had little or no effect on this antibody titer.

TABLE VII. RELATIONSHIP OF CHANGE IN REAGIN TITER TO CLINICAL RESPONSE

	NUMBER OF PATIENTS	REAGIN TITER		
		INCREASE	DECREASE	SAME
Pre-repository to pre-season				
Improved	9	3	2	4
Not improved	5	1	1	3
Pre-repository to post-season				
Improved	9	4	2	3
Not improved	4	1		3
Pre-season to post-season				
Improved	9	2	1	6
Not improved	5	1		4

3. There apparently was no relationship between the time the repository therapy was given and the change in reagin titer (Table VIII).

TABLE VIII. RELATIONSHIP OF TIME OF REPOSITORY INJECTION TO CHANGE IN REAGIN TITER

FOLLOWING REPOSITORY THERAPY (WEEKS)	NUMBER OF PATIENTS	REAGIN TITER		
		INCREASE	DECREASE	SAME
10	5		2	3
8	5	2	1	2
5	2	1		1
3	1			1
2	1	1		

4. Of these 14 patients who were so studied, 3 had an increase of fourfold or greater of their hemagglutination titer, but the reagin titer remained the same. Contrariwise, of the 11 patients whose hemagglutination titer remained unchanged, 4 demonstrated an increase in reagin titer, 3 decreased, and 4 remained the same (Table IX).

TABLE IX. RELATIONSHIP OF CHANGE IN HEMAGGLUTINATION TITER AND REAGIN TITER

HEMAGGLUTINATION		REAGIN TITER		
		INCREASE	DECREASE	SAME
Increase	3			3
Same	11	4	3	4

C. *Blocking Antibody.*—

1. Eight of these 14 patients showed an increase in blocking antibody. Three patients who received 500 pollen units, and 3 who received 10,000 pollen units, did not demonstrate an increase in blocking antibody (Table X).

TABLE X. REPOSITORY DOSAGE TO CHANGE IN BLOCKING ANTIBODY TITER

DOSAGE (POLLEN UNITS)	BLOCKING ANTIBODY TITER	
	INCREASE	SAME
500	0	3
1,000	3	
2,500	3	
5,000	2	
10,000		3

2. There, likewise, was no correlation between the clinical response and increase in blocking antibody. Five of the 9 patients who were clinically improved had an increase in blocking antibodies. Three of the 5 patients who were not benefited by treatment also showed an increase in blocking antibody (Table XI).

TABLE XI. RELATIONSHIP OF CHANGE IN BLOCKING ANTIBODY TITER TO CLINICAL RESPONSE

CLINICAL RESPONSE	NUMBER OF PATIENTS	BLOCKING ANTIBODY TITER		
		INCREASE	DECREASE	SAME
Improved	9	5		4
Not improved	5	3	1	1

3. There was no relationship between the time of repository injection and blocking antibody. However, patients who received the emulsion 2 or 3 weeks previously probably did not have time to develop more blocking antibodies (Table XII).

TABLE XII. RELATIONSHIP OF TIME OF REPOSITORY INJECTION TO CHANGE IN BLOCKING ANTIBODY TITER

FOLLOWING REPOSITORY THERAPY (WEEKS)	BLOCKING ANTIBODY TITER		
	INCREASE	DECREASE	SAME
10	3		2
8	3		2
5	2		
3		1	
2			1

4. Two of the 3 patients who had an increase in hemagglutination antibody titer, likewise, had an increase in blocking antibody. These 2 patients had satisfactory relief of symptoms; the other did not. Of the remaining 11 patients whose hemagglutinating antibody titer was unchanged, 4 had an increase in blocking antibody but only 2 had clinical relief of symptoms (Table XIII).

TABLE XIII. RELATIONSHIP OF CHANGE IN HEMAGGLUTINATION TITER AND BLOCKING ANTIBODY TITER

HEMAGGLUTINATION		BLOCKING ANTIBODY TITER		
		INCREASE	DECREASE	SAME
Increase	3	2	1	
Same	11	4		7

5. A comparison was made of the change in titer of skin-sensitizing antibodies and blocking antibodies following repository therapy. Of 4 patients who demonstrated an increase in reagins, 3 likewise developed a higher blocking antibody titer. However, the blocking antibody titer also increased in 3 patients in whom the reagin titer remained unchanged (Table XIV).

TABLE XIV. RELATIONSHIP OF CHANGE OF REAGIN TO BLOCKING ANTIBODY TITER

REAGIN TITER		BLOCKING ANTIBODY TITER		
		INCREASE	DECREASE	SAME
Increase	4	3		1
Decrease	3	2		1
Same	7	3	1	3

D. *Scratch Test Titrations.—*

1. *Pre-seasonal group of patients:*

A. Serial threefold dilution scratch tests were performed on 44 patients of this group prior to the injection of the repository pollen extract and again just prior to the ragweed pollen season. Twenty-eight of these 44 patients had a decrease in skin reactivity. However, there was no correlation noted between the amount of repository extract injected and the response to skin reactivity. Nine of 12 patients who received 1,000 pollen units had a decrease in skin reactivity, whereas 3 of 5 receiving 10,000 pollen units also reacted in the same manner (Table XV).

TABLE XV. DOSAGE TO CHANGE IN SCRATCH TESTS*

DOSAGE IN REPOSITORY THERAPY	SKIN REACTIVITY		
	INCREASE	SAME	DECREASE
500		1	3
1,000	2	1	9
2,500	4	2	6
5,000	2	1	7
10,000		2	3
20,000		1	1
Totals	8	8	28

*Pre-repository to pre-season.

B. There was no apparent relationship between the change in skin reactivity following repository therapy and clinical response. Of the 28 patients, whose skin reactivity was decreased, exactly one half clinically benefited from repository therapy. Likewise, of the 8 patients whose skin titers remained the same, an equal number was helped (Table XVI).

TABLE XVI. SKIN REACTIVITY TO CLINICAL RESPONSE

CLINICAL RESPONSE	SKIN REACTIVITY		
	INCREASE	SAME	DECREASE
Total number of patients	28	8	8
Total number improved	14	4	6

DISCUSSION

1. *Immunologic Response.*—

In previous studies[3] we have shown that 68 per cent of the patients receiving multiple injection treatment for ragweed pollinosis had at least a fourfold increase in hemagglutinating titer. In our present investigation, only 24 per cent of the patients treated by pollen emulsion (no matter what the dosage) had a similar type of response. Although the group of patients (38) who had only 3 to 8 small aqueous doses produced a greater antigenic response than did those on repository therapy, they did not show as great a hemagglutination antibody response as did those patients who received many aqueous injections over a longer period of time. This difference could conceivably be explained by the fact that there was a much smaller amount of extract, fewer dosages, and a shorter time interval to develop these antibodies.

This very low antigenic stimulation of hemagglutinating antibodies with the ragweed repository therapy in human beings is quite puzzling. Similar findings were reported by other investigators.[8, 9] One would anticipate a greater response on the basis of previous reports of emulsion therapy in humans with influenza virus vaccine[10] and in animals with multiple antigens.[11] Perhaps the explanation might be that either we did not use enough pollen extract in our mixtures or that the antigenicity of the ragweed extract was not comparable to more potent substances.

Although it is true that we did not find any correlation between the increase in hemagglutinating titer and clinical response in either our present or previous studies, the hemagglutination activity is, nevertheless, an indication of some type of antigenic response. Whether this type of response is important as far as the explanation of the mechanism of hyposensitization therapy is concerned is still in doubt. We also could find no correlation between the skin-sensitizing and blocking antibody titers to each other or to clinical response. Hence, if none of these antibodies play a role in relief of symptoms, either by multiple injection or emulsion therapy, how do these treatments work? It is conceivable that there are changes at the tissue (shock organ) level that we are, as yet, unable to measure and determine. Certainly, clinical experience has demonstrated, in the past 50 years, that hyposensitization therapy, although not perfect, is beneficial.

Although a good percentage of our patients did well on placebo therapy, this was not a good enough controlled study, as there may have been a carry-over from previous multiple injection therapy. It would be very difficult to explain all of these good results on psychogenic factors alone.

Hennessy and Davenport[12] have shown that with two injections of influenza virus emulsion in small doses at a 4 to 6 week interval there is a much larger protective antibody response developed than if only one large dose of influenza emulsion is given. Sheldon and associates[13] recently reported using this same technique with grass hay fever patients and claimed good clinical results. Unfortunately, there were no reported antibody studies. Perhaps, utilization and application of the two-injection small-dosage technique for pollinosis should be investigated further. Patients will have to be carefully selected with adequate placebo controls and careful observation. Additional serological studies of the various antibody responses should be determined. Likewise, a group of investigators, competent and critical, should treat patients in the exact manner, using the same materials, methods of preparation, and dosage schedule as described by Brown.[14] It is only by these methods, over a period of several years, that we will be able to establish whether pollen emulsion therapy is effective, safe, and free of subsequent complications.

SUMMARY AND CONCLUSIONS

1. The repository therapy did not produce as good an antigenic response (as determined by hemagglutination titers) as did multiple injection therapy.

2. There was no relationship found between the change in skin-sensitizing or blocking antibody titers and the clinical response or hemagglutinating antibody titer following repository therapy.

3. The need for more intimate knowledge of the mechanism of specific hyposensitization therapy is stressed.

4. Carefully controlled studies over a period of several years is still necessary to establish the true value and safety of repository therapy.

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DIPHENYLHYDANTOIN (DILANTIN) HYPERSENSITIVITY WITH INFECTIOUS MONONUCLEOSIS-LIKE SYNDROME AND JAUNDICE

Sheppard Siegal, M.D., and Jesse Berkowitz, M.D., New York, N. Y.

DIPHENYLHYDANTOIN (Dilantin) is a mainstay of antiepileptic treatment, and, in contrast with other anticonvulsants, such as Mesantoin and Tridione, it is generally considered a safe compound.[1] The purpose of this report is to draw attention to an uncommon form of allergic reaction induced by Dilantin. This is characterized especially by a hemopoietic response which simulates infectious mononucleosis, and by evidence of liver damage including jaundice. Dilantin hypersensitivity of this type has been noted by two observers in recent years.[2, 3]

CASE REPORT

H. L., a 34-year-old Negro housewife, was first admitted to the Neurological Service of the Mount Sinai Hospital on Sept. 8, 1958, because of a 2-week history of recurrent dysesthesias and impaired mobility of the left side of the body. Episodes recurred as often as 20 times a day, each lasting perhaps 30 seconds. There was no loss of consciousness, major seizure, or incontinence. General physical examination showed the liver to be at the costal margin. The sole findings on neurological review were a slight decrease in left arm swing and slight increase in deep tendon reflexes on the left side.

Hemoglobin was 12.2 grams and the white blood count 7,350 cells per cubic millimeter with a normal differential count. The cephalin flocculation test was 1 plus. The results of lumbar puncture and spinal fluid tests were negative. A pneumoencephalogram demonstrated slight dilatation of both lateral ventricles. The electroencephalogram showed diffuse slowing with focal accentuation in the right posterior temporal region. A right carotid arteriogram was within normal limits.

The neurological diagnosis was right cerebral dysfunction, cause unknown. On September 23, the patient was started on the anticonvulsant drug, diphenylhydantoin (Dilantin), and advised to continue it in a dose of 100 mg. three times daily.

On October 28, she was readmitted to the Medical Service at the Mount Sinai Hospital. Her complaints were of sore throat, chills, fever, and increasing lethargy of 8 days' duration. At onset she was seen in the emergency room where a penicillin injection was given, and oral sulfonamides prescribed. On the next day, large pruritic skin lesions and tender swollen cervical nodes were noted. At another hospital, tetracycline was substituted for the sulfonamide and Benadryl was also prescribed.

At the time of her second admission the patient looked acutely ill and lethargic. Her temperature was 104° F. There was generalized lymphadenopathy and the liver was palpable 2 cm. below the costal margin. The spleen could not be felt. There were no meningeal signs. A maculopapular erythema was most marked on the arms and chest.

From The Medical Services of the Mount Sinai Hospital, New York, N. Y.
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The hemoglobin was 11.6 Gm., the total white cell count 33,000 per cubic millimeter., of which polymorphonuclears were 45 per cent, lymphocytes 51 per cent, monocytes 2 per cent, eosinophils 1 per cent. Of the lymphocytes, 36 per cent were atypical forms as seen in infectious mononucleosis. In appearance they closely resembled those seen in mononucleosis. The heterophil antibody titer, unabsorbed, was 1:56 on October 30. The bone marrow was normal. Chest roentgenogram and electrocardiogram were negative.

The urine showed a trace of bile, urobilinogen positive in dilution 1:320, and a trace of albumin. The serum bilirubin was 2.0 mg. per cent; alkaline phosphatase, 28.3 King-Armstrong units; serum glutamic oxaloacetic transaminase, 300 units; total protein, 6.0 Gm. per cent; albumin, 3.2; globulin, 2.8. The prothrombin time was 14.5 seconds, control 12.0. Blood culture was sterile. Many L.E. cell preparations were made, including some to which Dilantin was added, but all were negative.

The admission diagnosis was infectious mononucleosis with jaundice. The skin eruption was considered to be unrelated and was attributed to penicillin allergy. Diphenylhydantoin (Dilantin) which the patient had been taking since her previous admission was continued.

The patient did poorly for the first 3 days with daily temperature rises to 104° F. Jaundice appeared on the fourth day and the total serum bilirubin was found to be 4.1 mg. per cent, of which 2.7 was direct reacting. The serum alkaline phosphatase had risen to 42 King-Armstrong units.

At this time, when her course thus far had been unfavorable, the diagnosis of hypersensitivity to diphenylhydantoin (Dilantin) was suggested as the most probable cause of the entire syndrome. The drug was therefore discontinued. The next day the temperature fell to 101° F., and in another 48 hours the patient became afebrile. Although at this time marked skin exfoliation became apparent, her general condition improved strikingly and the icterus subsided. A few days later, for a 48-hour period, she again had fever to 103° F. However, no new symptoms appeared, icterus did not recur, and both serum bilirubin and the glutamicoxaloacetic transaminase test remained normal. Without additional therapy, the fever again subsided and the subsequent course was of persistent improvement.

Subsequent blood counts showed a leukocyte count decreasing from 24,000 to 14,000. Lymphocytes and mononuclear forms accounted for from 45 to 60 per cent of the total. The abnormal forms were at lower levels than on admission, amounting to 13 to 25 per cent of the mononuclear cells. Eosinophils were more prominent than at first, reaching a maximum of 12 per cent. After one week the heterophil antibody reaction was negative after guinea pig kidney cell absorption. In another 2 weeks it was still only 1:14. Serum alkaline phosphatase activity remained high until 24 days after the discontinuance of Dilantin. Simultaneously the cephalin flocculation test which had been 4 plus fell to normal. Later values were again 2 to 4 plus but, unaccompanied by other evidence of liver damage appeared to be related to a rising total serum globulin. On November 10, serum electrophoresis showed an elevated gamma globulin. The white blood cell count reached 7,000, with 65 per cent polymorphonuclear cells and 28 per cent lymphocytes, and 7 per cent monocytes. The hemoglobin fell to 9.8 grams. The Coombs test was negative.

On November 10, the patient's serum was examined for precipitins to Dilantin by both ring tests and gel diffusion methods. False positive reactions were due to nonspecific precipitation of Dilantin, present also against control sera. Passive transfer tests with the patient's serum were carried out. Forty-eight hours after the sites were sensitized, the subject ingested 100 mg. of Dilantin without eliciting any local reaction at the sensitized sites. The sites were not tested directly with Dilantin solution as the drug is a local irritant.

On the morning of November 23 the patient felt well, lymphadenopathy had almost completely disappeared, and the skin had returned almost to normal. A single oral test dose of 100 mg. of Diphenylhydantoin was administered. Two hours later she developed generalized pruritus. At 6 hours she had a shaking chill with a temperature rise to 104.8° F. recorded 8 hours after the challenging dose of the drug. Soon thereafter the cervical lymph nodes were again found to be swollen and tender.

Twenty-four hours later the patient was again afebrile and the adenopathy had subsided. A total and differential white blood count examined at the height of the febrile reaction was normal, but after 24 hours the eosinophils rose to 19 per cent and remained elevated for 2 days. Five per cent atypical lymphocytes were again seen. The serum globulin increased to 5 grams after this acute reaction. There was, however, no alteration in serum bilirubin, alkaline phosphatase, or SGO transaminase (Figs. 1 and 2).

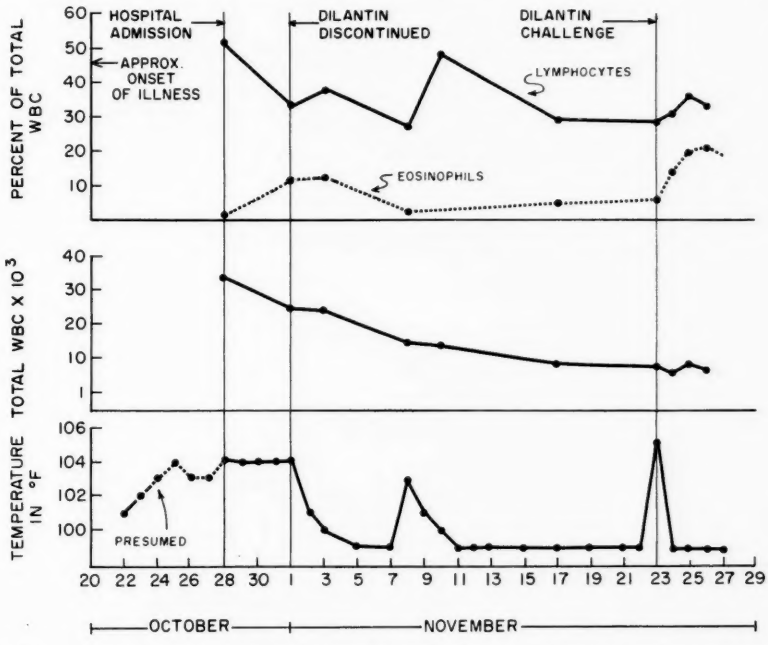

Fig. 1.—Febrile course and hematologic response in Dilantin hypersensitivity.

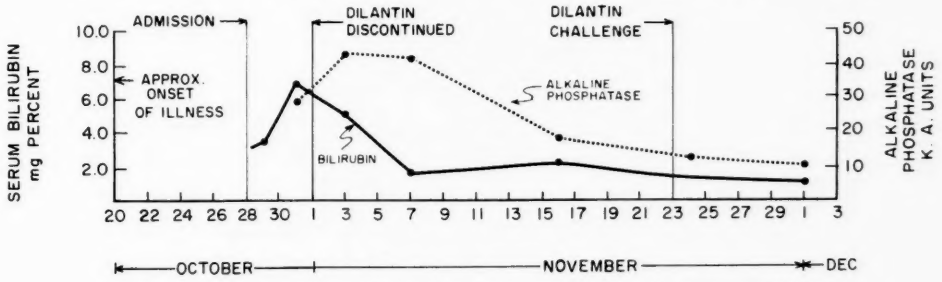

Fig. 2.—Bilirubinemia and hepatic damage in Dilantin hypersensitivity.

The morning after the patient's acute reaction to the challenging dose, serum was obtained for further serologic study. Complement fixation antibodies were sought not only against Dilantin alone as the antigen but the serum was also tested against incubated mixtures of Dilantin with desoxyribonucleic acid, with nucleoproteins, and with liver and kidney extracts. All complement fixation tests were negative. These studies were made at the Protein Metabolism Laboratory of the Rockefeller Institute.

By the time of discharge the patient was clinically well, the peripheral blood smear was normal, and there was no evidence of hepatic abnormality. Further treatment was carried out in the Neurological Clinic where she was started on both Mysoline, a non-hydantoin antiepileptic, and phenobarbital. This combination of medications was found effective and induced no allergic response.

DISCUSSION

Milder reactions to diphenylhydantoin (Dilantin), consisting of fever and a morbilliform or scarlatiniform eruption were reported soon after its introduction into clinical medicine. Although the drug is usually stopped when such reactions occur it has often been found possible to resume therapy without their recurrence.[4] However, after more severe reactions, especially when accompanied by exfoliative dermatitis, the drug cannot be reintroduced.

Chaiken, Goldberg, and Segal[2] described an instance of exfoliative dermatitis with accompanying lymphadenopathy and evidence of hepatic damage, with clinical icterus. An infectious mononucleosis-like blood picture was present with 67 per cent lymphocytes, of which 24 per cent were atypical forms. Gropper[3] also reported such a syndrome induced by Dilantin but his patient unfortunately died in hepatic coma. At autopsy widespread hepatic necrosis was found. Earlier, Mandelbaum and Kane[5] had observed exfoliative dermatitis with fever and hepatosplenomegaly but without the presence of either a lymphoid blood smear or jaundice. Perhaps reference should be made to the fact that periarteritis nodosa has also been observed as a rare sensitivity manifestation to Dilantin.[6]

Mesantoin, another hydantoin compound, has likewise been shown to cause a response of atypical lymphoid cells simulating infectious mononucleosis.[7] This tendency to intense stimulation of the lymph nodes as part of allergic reactions to the hydantoin anticonvulsants is further underscored by the recent extensive study of Saltstein and Ackerman.[8] In response to these drugs, a marked lymphadenopathy occurs of such degree as to mimic the malignant lymphomas clinically and pathologically. Peganone, 3-ethyl, 5-phenylhydantoin, is a newer compound incriminated in this type of allergic reaction.

Chemically Dilantin and Mesantoin are closely related to each other and also to Nirvanol. Dilantin is diphenylhydantoin. Nirvanol is phenylethylhydantoin. Mesantoin differs from Nirvanol only in having a methyl group substituted for a hydrogen group in one of the two NH groups of the hydantoin ring structure. Nirvanol, it will be recalled, once used in the treatment of rheumatic chorea, was frequently found to induce a syndrome referred to as "Nirvanol sickness." This was an allergic reaction which could be induced in over 80 per cent of the patients given this drug.[9] In addition to fever and an exanthematic eruption, lymphadenopathy was prominent, although atypical lymphoid cells were not described and jaundice was absent. Substitution of an ethyl for a phenyl group is the sole difference between Nirvanol and Dilantin, yet this relatively small chemical alteration results in a compound which is a much more frequent sensitizer.

It is also of interest that the hypersensitivity syndrome here described of pseudo-mononucleosis and hepatic damage may also occur in response to an unrelated compound, para-aminosalicylic acid,[10] one of the major anti-tuberculosis drugs. In para-aminosalicylic acid sensitivity, a positive patch test may be elicited by an ointment containing this drug. A comparable test with

Dilantin ointment was not attempted in our case as all further exposure to the drug was refused by the patient, because of the unpleasant reaction to the challenging oral dose.

In the differential diagnosis between true infectious mononucleosis and the hypersensitivity reaction which simulates it, probably the single most important diagnostic criterion is the heterophil antibody reaction. In Dilantin allergy, this test remains either negative or distinctly below the diagnostic titers which characterize infectious mononucleosis, usually a dilution of 1 to 120 or more. The more selective test carried out after absorption with guinea pig cells, specific for true infectious mononucleosis, is almost invariably negative in cases of drug allergy. Clinically a minor point of differentiation is the proneness to an extensive skin eruption in drug allergy whereas infectious mononucleosis is usually not attended by more than a minor erythema. The pronounced evidence of hepatic damage occurring relatively early in the course of the allergic reaction is also less common in the true infectious disease.

SUMMARY

An instance of an allergic reaction to diphenylhydantoin (Dilantin) is described which was attended by a peripheral blood picture simulating infectious mononucleosis and by hepatic damage with clinical jaundice. After recovery, re-administration of a 100 mg. dose of the drug induced acute fever, lymph node swelling, and eosinophilia. Efforts to demonstrate sensitivity to Dilantin by means of passive transfer tests, precipitin reactions, and complement fixation tests were unrewarding.

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THE CHANGING CONCEPTS OF ALLERGY

Harry L. Alexander, M.D., St. Louis, Mo.*

IT IS, indeed, gratifying to have been accorded the honor of having been asked to give the first Robert A. Cooke Memorial Lecture. Inasmuch as Dr. Cooke and I had been friends for a very long time—over 40 years—it is tempting to speak of him as a friend or as an individual, but it would seem more fitting to this occasion to attempt to portray the important role that he played, both in the establishment and in the development of clinical allergy. Not only was he a pioneer in this field, but the first half century of its existence fell within the span of his medical lifetime. Since many of his important contributions were made in the earlier years, I shall dwell briefly on this period.

Clinical allergy made its debut in June, 1911, in London with the publication in the *Lancet* of an article by Dr. Noon entitled "The Prophylactic Inoculation Against Hay Fever." Whereas there had been numerous published expressions of human hypersensitivity previous to this time, they had dealt largely with serum sickness, anaphylactic shock, and drug idiosyncrasies. Noon's communication was the first that introduced conventional concepts. He made a watery extract of grass pollen; he standardized this by an arbitrary unit of weight, tested patients with it by instilling the extract into the conjunctival sac, gave inoculations with increasing doses and secured successful results. It is of interest that Noon's concept of this clinical experiment was based on a fallacy in that he believed that hay fever was caused by a pollen toxin. This was the prevailing understanding of that day. He interpreted the positive conjunctival reaction as a sensitivity to the toxin and surmized that inoculation of this material would produce an antitoxin.

The treatment of hay fever by injection of pollen extracts apparently occurred to several individuals about this time, for, whereas Noon gave inoculations first in the autumn of 1910, Cooke was then designing his experiments, and Koessler was also engaged with the same problem. Within a few years, a dozen or more reports of treatment with pollen extracts appeared in the literature, both here and abroad. The successes obtained were attributed, largely, to the presumable production of an antitoxin.

Cooke presented the results of his experience before the section of Rhinology and Laryngology of the New York Academy of Medicine late in the year 1914.

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*Emeritus Professor of Clinical Medicine, Washington University Medical School, St. Louis, Mo.

He had accumulated more than 200 cases, the largest series reported up to that time. In this communication, he presented several original observations: the influence of heredity on the incidence of hay fever, the introduction of the intradermal method of skin testing, a more accurate method of standardizing extracts by determining the nitrogen content, a better way of preparing extracts, and he supported the concept, proposed earlier by Wolff-Eisner, that hay fever was caused by a nontoxic element in pollen. Cooke's remarks were published in abbreviated form in *Laryngology*, a specialty journal, in 1915, but they did not receive much attention.

In 1916, Cooke and VanderVeer's classic monograph on the treatment of hay fever with pollen extract appeared in the first volume of the *Journal of Immunology*. It contained abbreviated protocols of over 600 cases and confirmed Cooke's earlier observations. Again, it supported Wolff-Eisner's theory that the antigenic fraction of pollen extract is not a toxin, proof of which was supplied the following year by Cooke, Flood, and Coca through critical animal experiments.

In 1919, Cooke established one of the first allergy clinics—at the New York Hospital. This was at a time when both clinical and experimental research in allergy began to expand and, historically, the following decade was the most rewarding in this regard. Investigators, too numerous to mention here, recorded fundamental observations, so that by 1930 solid foundations of allergy had been laid. Although these have been added on to, little has been rebuilt. Cooke and his associates contributed substantially, for between the years 1922 and 1929 they published 36 separate articles under the designation "Studies in Human Hypersensitiveness," many of which were of considerable importance.

It was in these years that the first two national allergy societies were founded: The American Association for the Study of Allergy, and The Society for the Study of Asthma and Allied Conditions. Since Dr. Cooke was very instrumental in establishing the latter, I shall say a word about its first meeting. This was held in Dr. Cooke's office and among the twelve present was Dr. Warfield Longcope, Professor of Medicine at Johns Hopkins, who had done significant research on various aspects of human hypersensitiveness, and was the first to suggest that this be identified with allergy rather than with anaphylaxis. He was assisted by an able young investigator, Francis Rackemann, who also was present as were Dr. Oscar Schloss, Professor of Pediatrics at Cornell who had made earlier important observations on hypersensitivity in children, Dr. Coca, editor of the *Journal of Immunology*, soon to become Professor of Immunology at Cornell, and Dr. VanderVeer who did some early independent research. Dr. Cooke was elected president, I was secretary, and the dues were established at two dollars a year, an historic event. It was in 1929 that the first issue of the JOURNAL OF ALLERGY appeared.

By this time, clinical allergy was unable to keep pace with experimental advances, for there were very few experienced allergists and the membership of the national societies was small. Moreover, other than at annual meetings, there was no medium for communication among allergists until the JOURNAL OF

ALLERGY appeared. Meanwhile, allergy became increasingly popular among both physicians and the laity and there were not enough facilities to meet the demand for the elaborate skin testing in vogue at the time. As a consequence, early in the 1930's, specialists with scant training made their appearance and allergy clinics sprang up which they manned. This situation led to poor practice and extravagant claims. It should be said, however, that there was a solid core of competent allergists at the time who were making significant contributions. The existing situation was a matter of discussion in the councils of the national allergy societies and the consensus was that some way should be found to attract young physicians with good training to this specialty. However, in 1935, there were but 35 allergy clinics approved for training by a joint board of standardization of the two societies.

In 1936, the American Board of Internal Medicine was established for the very purpose of encouraging physicians to spend postgraduate years in disciplines that would enable them to pass examinations. After this, they would be certified as to their competence. Such a program seemed exactly suited to the situation in allergy, and application was made for subcertification which had been granted to other specialties of internal medicine. The request for subcertification was made jointly by the two national societies, and Dr. Cooke was chosen as spokesman. He was in an ideal position for this assignment for by this time he had acquired international stature. It should be remembered that he was primarily an internist with sound training. Before he concerned himself with allergy, he became interested in metabolic diseases and has several publications on the subject to his credit. His reputation as an internist is testified to, among other things, by membership in the Association of American Physicians where he became well known by those prominent in academic medicine; he became Governor, then Regent, and then Vice President of the American College of Physicians. Since 1932, he had been Director of the Department of Allergy at the Roosevelt Hospital in New York which later was to become the Institute of Allergy. I have a list of those who came to study with him since 1935. There are 45 names of physicians from seven countries and the list contains many of the outstanding allergists today. He was on important national committees and had received many honorary awards. With such distinguished sponsorship, certification in allergy was granted and Dr. Cooke was the first chairman of the subspecialty board.

Unfortunately, subspecialization in allergy failed in its purpose, since very few candidates have presented themselves for examination—some 4 or 5 each year on an average, exclusive of those who applied for certification in pediatric allergy. This mere handful cannot compare with the score or more certified annually in the more popular specialties. This lack of interest in allergy by graduate students is reflected also in the fact that there has been frequently a paucity of candidates to fill attractive fellowships offered by some of the outstanding allergy clinics. The fault here has not been with the subspecialty boards, and I feel sure that were there a separate board of allergy, which had been urged by some, the result would have been no different. If an under-

graduate or graduate student wishes to spend extra years in order to pursue some particular interest, either he must be sufficiently exposed to it or receive inspirational teaching.

In this regard, allergy has had little chance for, with few exceptions, this subject has not had a favorable position on the teaching curricula of most medical schools. As early as 1921, replies to a questionnaire sent to 35 schools indicated that there was little formal training in allergy and, in some instances, none. A similar response some 30 years later was received from many more schools. I do not know why this was so except in some of the leading institutions. Here, Department heads indicated that there was no lack of interest in the teaching of allergy, but rather a lack of teachers. This attitude did not imply that allergists could not teach, but that they were unprepared to teach this subject in a way that would conform to the curriculum.

It was believed that the allergist was too highly specialized in a restricted area of hypersensitivity. It was pointed out, for instance, that a second year medical student in the course in pathology is exposed to the lesions of allergy in their many aspects without emphasis on those of atopy; and, in immunology, skin sensitizing antibody is but one element of the underlying mechanism of hypersensitivity. With this background, when the student enters his years of clinical instruction, he should be taught allergy in its widest aspects. Although allergists experienced in asthma and hay fever were available, they were scarcely utilized.

It is true that, until more recent years, there were very few allergists who were equipped to teach allergy in its over-all aspects, and there were not many organized courses in this country where one could receive such comprehensive instruction—even in the schools that demanded it. It should be said, however, that the concept of the allergist as one who was limited in his knowledge of hypersensitivity was by no means correct, for medical periodicals contain innumerable articles on all aspects of the subject written by allergists, and these date from the very beginning of clinical allergy. Certainly, the allergist has broadened his interests in recent years and two examples of this may be cited. One occurred at the International Congress of Allergology in Paris in 1958, when the large amphitheater was filled, seemingly to overflowing, by allergists from all over the world, when symposia on immunology and on histamine were given. Again the program of this meeting and, particularly, the large attendance during the first 2 days when fundamental aspects of hypersensitivity were presented testify eloquently to the unrestricted interests of the allergist.

Fortunately, there has been a decided change for the better in instruction of allergy during recent years. Aside from a small number of medical schools where sections of allergy have been on a full-time status, there has been an increasing awareness of the number of diseases and other situations that are based on immune mechanisms. These are exemplified by the newer immunological concepts of hematology, the additions to autoimmune diseases, and the implications of immunological tolerance. Immunological techniques as measuring tools are being developed, and from such methods one may now detect, with

accuracy, incredibly small amounts of circulating hormones. Of the clinical disorders that are mediated by immune mechanisms, allergy is of the first importance. As a consequence of this new development, clinical immunologists, usually internists oriented to this specialty, have been added to Departments of Medicine. In some schools they have administrative control over the teaching of clinical allergy. In the few instances in which the immunologist is an experienced allergist, the situation is ideal, for allergy is taught in its proper perspective. Otherwise, allergists are called upon. The important point is that, no matter what method is used, at last the undergraduate student is beginning to receive proper instruction in allergy.

Another way in which allergy is finding a proper place in medical teaching is through the graduate teaching programs of the National Institute of Allergy and Infectious Diseases. When this Institute was established but a few years ago, certain categories were set up for the purpose of reviewing applications for research grants. One of these was "Allergy and Immunology." Here, again, is an exposition of the broad concept of integration of these two subjects. It was soon realized that there were few organized facilities for training in this field at a post-graduate level. Money was then requested from the Congress for this purpose, as well as for training in some other disciplines. Some $400,000 was allocated to Allergy and Immunology, and several medical schools welcomed the opportunity to teach allergy in terms of its broad implications. Dr. Karel will talk about these programs tomorrow, but I would mention that, if I am correct, in the fiscal year 1960, some $1,200,000, three times the initial amount, was spent for training and 28 medical schools participated. Applications from others have been approved and are awaiting more funds.

With this accelerated teaching of allergy on a broad level, we might inquire what our role as clinical allergists will be in this changing order of things. To begin with, as long as asthma and hay fever persist, we will be indispensable. Moreover, we will be essential to the teaching programs insofar as their clinical aspects are concerned. As trainees complete their courses, there will be many places awaiting them in academic medicine to teach allergy according to the concepts in which they have been trained. Eventually, many will drift into clinical practice, and these will be the allergists of tomorrow. I dare say, however, that as our interest in the wider aspects of hypersensitivity increases, we all shall speak the same language. Since the trainees are exposed to basic research during their courses of instruction, some may approach clinical allergy with an investigative attitude and concern themselves with the many unsolved problems that await attention, and this is good.

As advances are made, some of them doubtless will be reflected in this lectureship, perhaps for years after the personal recollection of Robert Cooke has disappeared. His name, however, will hereby be identified with progress in allergy, just as it was during his lifetime, and this is as it should be.

EDITORIAL

ALLERGY ABSTRACTS

Readers of the Journal of Allergy have perhaps come to take for granted the appearance in each issue of some thirty abstracts covering articles of direct or possible interest to them. In addition to asthma, hay fever, and anaphylaxis, fields covered include dermatology, otorhinology, ophthalmology, pharmacology, physiology, and pathology. Members of the Editorial Board are former residents of the Allergy Division of the Jewish Hospital of Brooklyn, New York. Wide coverage of publications in the field of allergy and related subjects is achieved in the course of each year. This is no small task and presents an ever recurring challenge to the editorial Board and its collaborators.

Allergy Abstracts first appeared in 1936 as a privately circulated publication at a time when the Journal of Allergy was in its seventh year. Beginning in 1934, the Journal had carried a Department of Reviews and Abstracts which to some extent paralleled Allergy Abstracts. In 1944, the year in which the American Academy of Allergy emerged from the fusion of the Eastern and Western societies, publication of Allergy Abstracts in the Journal of Allergy was initiated, a happy arrangement which continues to the present day.

It is a pleasure in this, the twenty-fifth year of Allergy Abstracts, to acknowledge our debt and gratitude to the Editor, Dr. Murray M. Albert, and to his able Editorial Board and assistants. It is to be hoped that the symbiotic relationship between the Journal and Allergy Abstracts will continue to flourish in the future as it has in the past.

F. C. L.

AMERICAN ACADEMY OF ALLERGY

REPORT OF THE AIR PROCESSING COMMITTEE FOR 1960

A REPORT by this committee in 1956 discussed the general principles involved in the selection of an air cleaning device. Since that report was published, many new "air cleaners" and "air purifiers" have come on the market. Some are good and others have little if any value. Some may even be detrimental to health if used under certain conditions. A fresh look at the problem seems indicated at the present time.

Clinical studies,[2-5] done on patients with pollen hay fever between 1930 and 1936, demonstrated that many of these patients were relieved of their hay fever a few hours after they were placed in rooms which were equipped with devices which filtered large amounts of pollen from the air within the rooms. Most of these patients stayed free of their hay fever as long as they remained in the room. Pollen asthmatics in general were much slower in responding to the relatively pollen free air of the test rooms, but many obtained benefit after one to 3 days of confinement in such rooms. In 1936, Criep[6] described the first room unit two-stage electrostatic air cleaner. He used this unit to clean the air of pollen and other particulate matter within hospital rooms in which allergic patients were confined. He stated in his conclusion, that, "A study of sixty-one patients seems to indicate that the electrostatic cleaner has a place in the treatment of certain refractory cases of hay fever, pollen asthma and bronchial asthma. Hay fever and pollen asthma patients for the most part experience symptomatic relief for the period during which they are confined to the air cleaned room."

These studies demonstrate rather clearly in the cases of patients who are sensitive to ragweed that the pollen content within a given room can be lowered by: (a) filtering the incoming air through a high efficiency filter, and (b) filtering the air through a low efficiency filter, providing the air in the room is recirculated through the filter at a fairly rapid rate (example, use of window air conditioner with no filter).[2, 3]

It has been estimated that there will be a complete change of air about every hour within a closed room due to leakage of air through the cracks around windows and doors. This hourly factor will vary somewhat with the outside air movement. It should be obvious that a filtering device which moves 20 cubic feet of air per minute through its filter would not even keep up with the

expected change of air in a room 12 by 14 by 8 feet, since such a room contains 1,350 cubic feet of air and the filter would be moving only 1,200 cubic feet of air every hour.

It would seem that a room air cleaner, in order to be effective as a recirculating filtering device, should move at least 100 c.m.f. Such a device should have a very high efficiency filter. For a low efficiency filter (60 per cent), it would be desirable to move much more air, say 200 to 300 c.m.f., in order to do a good job of pollen filtering.

Window filters which bring all the air from the outside and do not recirculate room air must of necessity contain a highly efficient filter—95 per cent or better. Such window filters are still manufactured and rely upon a five-ply paper filter of large area.

The determination of the efficiency of a given air filter requires: (1) an understanding of certain basic principles, and (2) highly technical equipment. It is possible to test a filtering device during the ragweed season by placing slides, coated with petroleum jelly, upstream and downstream from the filter, and counting the number of pollen grains per field on the two slides. Such a method, in order to be at all accurate, would require the construction of identical tunnels for both up and down stream sides of the filter, in order to ensure that the rate of air flow past each slide is the same. Out-of-season tests of this kind would require some method of feeding pollen at a uniform rate to the upstream side of the filtering device.

Most of the large companies who manufacture air filtering equipment have constructed wind tunnels in which air can be driven through the filter under test at the filter's rated capacity. They have various methods of feeding pollen to the intake side at a uniform rate. They use a highly accurate sampling technique of drawing a known quantity of upstream air through a Millipore filter and counting several fields. On the downstream side, many times the amount of air is drawn through a similar Millipore filter and again an average count of several fields is determined. In this manner the true efficiency of a filter can be determined with relation to ragweed pollen.

By this method it has been determined that: (1) five-ply "air mat" filter paper is 95 to 97 per cent efficient, (2) ordinary glass wool impingement filters are 60 to 75 per cent efficient, (3) certain very small-fibered glass wool impingement filters may be up to 95 per cent efficient, (4) electronic (two-stage electrostatic) filters are 99.5+ per cent efficient, and (5) the ½ inch glass rug filters, which are manuafeteured with glass fibers of approximately 2 μ in diameter, are 100 per cent efficient, but require much more power to overcome their resistance to air passage. The above figures are correct provided rated capacities of filters are adhered to.

The allergist's interest in air filtration does not stop with ragweed and other pollens. He has patients who are clinically sensitive to animal danders, house dust, molds, dusts which are found in grain mills, and some other inhalants. Certain mold spores, much of the house dust, portions of the animal danders, and some other dusts present a peculiar problem in so far as air

filtration is concerned because many of these particles fall into the range of 0.2 μ to 2.0 μ in diameter. Ordinary paper and impingement filters are only about 10 per cent efficient for small particles. There are only 3 filters which are effective in removing high percentages of such particles: (1) the "absolute filters," (2) the glass rug, ½ inch or thicker, and (3) the electronic filter (two-stage electrostatic). There is a variation of the electrostatic filter made which consists of a loose glass fiber on which are printed carbon circuits, which circuits have a potential differential of 2,000 to 3,000 volts DC. This filter is less effective than a good two-stage electrostatic filter, but is somewhat effective in the removal of the particles 0.3 to 2 μ in size.

In order to test filters for their efficiency in this size range of particles, the Air Filter Institute[7] has adopted the National Bureau of Standards Atmospheric Dust Spot Test. Members of the American Society of Heating and Refrigerating Engineers (A. S. H. R. E.) also use this method with some reservations. The main objection to this test is that there is about a 2.5 per cent variation day to day in one city, and perhaps as much as 10 per cent variation between cities—thus making it difficult to be highly accurate in evaluating the efficiency of competing products.

The British National Standard is the methylene blue test,[8] in which a solution of 1 per cent methylene blue dye in distilled water is aerosolized into the upstream side of the filter under test. The droplets evaporate almost immediately and the dye is dry by the time it arrives at the filter face. The particles of dye range from 0.1 to 1.0 μ. This method has the advantage over the dust spot test in that it can be done the same way in any location and is not subject to weather factors. It is, however, a fussy test. The solution must be fresh, the nebulizer must be correctly made, the orifices must be kept clean, and the dye must be carefully filtered just before nebulization. Perhaps within a few years some other method will replace both it and the dust spot method. The methylene blue test, when done properly, does compare very closely with the dust spot test, and both offer a fairly accurate picture of what happens to small dust particles and mold spores in a given filtering device.

There is one new two-stage electronic room air cleaner which has come on the market during the past year which processes about 100 c.m.f., is better than 99.5 per cent efficient in its ability to remove ragweed pollen fed into it, and is said to be 90 per cent N.B.S. dust spot efficient. Another one is said to process about 200 c.m.f. and still have the same efficiency. These retail at a little over $200. The industry is likely to produce others within the next year or two.

There is one single-stage electrostatic filter being marketed which processes 130 c.m.f., is 99+ per cent efficient on ragweed pollen, but is less than 50 per cent dust spot efficient.

Several large air filtering companies have been making highly efficient two-stage electronic air cleaners for the commercial market for many years. These are all well constructed and of good design. They are all highly efficient

for pollens, 99+ per cent, and if installed properly and at rated capacity are 90+ per cent N.B.S. atmosphere dust spot efficient. Their dust spot efficiency will drop if more air than the rated capacity is driven through them.

Smaller models of these commercial type air cleaners have been available for large homes for many years, but it is only within the past 5 years that the industry has turned its attention to the smaller homes.

Today it is possible, in a 1,200 square foot home, to install for $200 to $300 a good two-stage electronic filter in the forced air heating system. These units are of different sizes and shapes. One is being built into an outer steel casing which is designed to fit onto the furnace. Its collecting plates may be easily removed as a unit and cleaned with special detergent and hot water. Another one is similar except that it has to be installed in the duct, is 5 inches thick, and is also removable so the plates can be washed clean. Another one is only 2 inches thick. This one can replace the ordinary 2 inch impingement filter, and may thereby require no installation cost except the necessary wiring to its power pack. This model, however, has a throw-away collector grid and filter which will need replacement one or twice each year. All of these units will test out at 60 to 70 per cent dust spot efficient. This is not as good as the larger commercial and semi-commercial units, but when one considers the difference in cost and the fact that these are installed in a recirculating system they are to be given real consideration if the resident of the home is allergic to pollens, molds or house dust. It will be obvious that when the temperature outside is 90° F. or higher, air conditioning, in addition to the electrostatic filter, will add to the comfort of the occupants and will at the same time make it possible to keep the windows sealed and the outside doors closed except for ingress and egress. Even an electronic filter will be ineffective if windows or outside doors are left open.

During the past few years a new class of device has come on the market. Generally speaking, their advertising matter states they assure "wonderful relief[19] for hay fever and asthma," and eliminate odors even in closed rooms. One such machine was recently looked at by this committee. It contained a small two-bladed fan which pushed air through a small standard glass wool impingement filter after the air was subjected to the emanations from 3 four-watt General Electric ozone lamps. This device was found to move a little less than 20 cubic feet of air per minute. Since the filter was known to be less than 75 per cent effective in its ability to remove ragweed pollen, it became evident that this combination of filter and speed of air movement could hardly be expected to produce any appreciable effect on the pollen content of room air unless the room was of closet size.

It has been stated that, "Ozone has no therapeutic value and is detrimental to living beings long before concentrations are reached which have any bactericidal or deodorizing value."[19] Since the above-mentioned device had no other deodorizing component it would appear that its ability to eliminate odors depended on a physiologic principle rather than any actual reduction in odor. It had been observed that a person who enters a smoky

room in which ozone is present will not notice the tobacco smoke after he has been in the room for a few minutes. The effect of ozone on the olfactory apparatus makes it refractory to other odors.

This committee was fortunate in obtaining cooperation from the Los Angeles Air Pollution Control District (A.P.C.D.) in determining the ozone producing capacities of this and several other devices. A double-sized unit was placed in a glass-walled 12 by 10 by 8 foot room and turned on for 15 hours. The 6 four-watt ozone lamps, at the end of 15 hours, had driven the ozone content of the room up to 0.53 parts per million. When the ozone in outside air in Los Angeles rises to 0.5 ppm, this level constitutes a "first stage smog alert." The American Medical Association Archives of Environmental Health[10] has specified that a workman can be subjected to no more than 0.10 ppm of ozone during an 8-hour working day, this despite the fact that ozone can be detected by the human nose at about the 0.03 ppm level. In view of this machine's ability to produce relatively high ozone concentrations it would seem desirable to use some other method of odor elimination.

There is no better way of eliminating bad smells within the home or office than to eliminate the source, open the window, and air the place out. There is, however, another method of odor and gas elimination which does in fact remove bad smells. This entails the use of activated charcoal which has a high affinity for many gases above the molecular weight of oxygen, and most odors, including ozone. It has been used successfully in atomic submarines which have stayed submerged for months. It has been successful in eliminating Los Angeles smog from a number of buildings and homes. In order for this method to be highly effective the air must pass through a layer of pea-sized activated charcoal particles.

It should be noted that ozone can be produced in a number of ways. Ionizing wires used in two-stage electronic air cleaners, if incorrectly spaced so that a corona results, will produce ozone, as will arcing across bent collector plates in these filters. The A.P.C.D. tested one single-stage electrostatic filter and it produced 1.5 ppm of ozone in 15 hours. A small change in the ionizing rods and a different power pack reduced its ozone production to 0.02 ppm. One room type two-stage electronic air cleaner was tested and produced only 0.02 ppm. These tests are mentioned because an electrostatic air cleaner producing enough ozone to be detected should first have its plates cleaned and, if this is ineffective, the unit should be inspected for damage.

This brings us to the final problem which is likely to plague the allergist until more critical and carefully controlled studies are done—namely, negative ionization.

Negative Ionization.—In an article entitled "Air Ion Density and the Allergic Individual" by Corrado and Beckett,[11] there is a review of certain theories of air ion production. The article also reviews some of the published work of Kornblueh and gives references to this work. It also mentions Krueger's initial paper on the effects of negative and positive ions on ciliary activity in rabbit tracheal mucous membrane. The article "Some Biologic

Properties of Gaseous Ions" by Krueger[12] states that, "Our experiments with the responses of the trachea to inhalation of air ions demonstrated that negatively charged oxygen and positively charged carbon dioxide are the physiologically active components of ionized air. In general, negatively charged oxygen stimulates the mucosal surface—clearing mechanism; positively charged carbon dioxide inhibits it and renders the tissue markedly vulnerable to trauma."

This is the latest of a series of seven papers published on this subject by Krueger and Smith.[12-18] In reviewing all of the literature on this subject it would appear that the work of Krueger and Smith merits rather careful scrutiny. If this work is confirmed, it would appear that the allergists should under very carefully controlled conditions re-evaluate the clinical effects of negative versus positive ionization in hay fever and asthma.

One of the major difficulties encountered in doing such an evaluation would be that of determining accurately the degree of negative or positive ionization present in the patient's environment. In this connection it should be noted that the ionizing unit used in Krueger's work was placed inside of a glass or plastic box and pointed directly at the rabbit's exposed trachea. If the Krueger drawings are correct it would appear that the box would contain not over 2 cubic feet of space and that the ionizing element is not over 12 inches from the tracheal mucous membrane. This is the identical unit which is being marketed for use in a room inhabited by the patient. If such a room contains 1,000 cubic feet (a small room) one could postulate that the human being would receive only one five-hundreth the number of negative ions which bathed the rabbit trachea in Krueger's experiments.

The Beckman Instrument Company makes a micro-micro ammeter which, with supporting apparatus, is capable of measuring ions, but the total cost of the needed four such instruments, plus the recorder, runs close to $10,000.

It is possible that some simpler and less expensive method will be devised to determine the number of negative and positive ions per cubic centimeter of air. Some such measuring device should be used if accurate clinical studies are to be done.

SUMMARY

The National Bureau of Standards dust spot atmospheric air test and the methylene blue test for determining filter efficiencies to the 0.1 to 2.0 μ particles are discussed.

The newer and less expensive electronic air cleaners for installation in existing or new forced air heating and air-conditioning systems are reviewed.

The electronic portable air cleaners are reviewed.

The so-called air purifiers are discussed.

The possibility that negative ionization may have some bearing on the allergic patient is mentioned.

Respectfully submitted,
Willard S. Small, M.D.
Chairman

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AMERICAN ACADEMY OF ALLERGY

REPORT OF COMMITTEE ON CONTACTANTS FOR 1960

I. REGISTERING OF NEW CONTACTANTS

Dr. Rudolf L. Baer, of New York City, through Dr. Norman L. Kanof, suggests inclusion of the following in a list of patch-test materials: (1) dihydroxyacetone—4 per cent aqueous solution (this is found in cosmetics, such as Man-Tan), and (2) polyethylene plastic—as is.

Dr. Donald J. Birmingham of Cincinnati will submit shortly some recent information and remarks on Man-Tan and on celery dermatitis. It has been established dihydroxyacetone is the sensitizing chemical in Man-Tan.

Dr. George H. Curtis of Cleveland submitted the following report, obtained in response to an inquiry to members of the Cleveland Dermatology Society: Dr. George M. Stroud reported 3 cases of contact dermatitis due to bithionol, U.S.P., brand name Actamer (Monsanto Chemical Company). The testing concentration is 2 per cent in ethanol. It is used for its bacteriostatic or antiseptic action, and is related to hexachlorophene. It is present in J. & J. first aid cream, Clesk (bar soap), and liquid soap for surgical scrubs.

Dr. Morris Leider of New York City called attention to undecylinic acid and its zinc salt as a cause of allergic eczematous contact dermatitis.

II. RESEARCH

Dr. Rudolf L. Baer and staff have reported in the *Journal of Investigative Dermatology* and *Archives of Dermatology* studies in drug photosensitivity and attempts to transfer eczematous contact type allergy with whole blood transfusions.

Dr. Otis F. Jillson of Hanover, N. H., although not a member of the Committee, was asked to offer some comments on his studies of solar dermatitis. Photo skin testing, employing a carbon arc light with special attachment, is used in all cases of suspected solar dermatitis. Photocontact dermatitis is caused by a variety of drugs (phenothiazines, newer oral sulfonamides, declomycins) cosmetics, and plants. The photoskin test helps distinguish between phototoxic and photoallergic reactions.

III. RELATIONSHIP WITH FOOD AND DRUG ADMINISTRATION

Dr. Irvin Kerlan of Washington, D. C., pointed out the recently enacted Federal Hazardous Substances Labelling Act. This statute deals with the hazardous properties of substances found in the household, excluding foods, drugs, cosmetics and economic poisons (Public Law 86-613, 86th Congress, S.1283, July 12, 1960).

MISCELLANEOUS INFORMATION

Dr. Alex S. Friedlander of Detroit, Michigan, called attention to the study by the Division of Occupational Health of the problem of "celery dermatitis" among celery farm workers in the state of Michigan. This body found that there were two causes, namely pink-rot, a fungus infection of the plant, plus photosensitivity. The use of a sun-screening cream seems to be encouraging in the prevention of this distressing condition. The report was taken from the Michigan State Board of Health Column which appeared in the *Journal of Michigan State Medical Society* (9: 1424, 1959).

Dr. Herbert H. Johnson of Cleveland noted the occurrence of sensitivity to protective creams used in industry to prevent reactions to known irritants.

Respectfully submitted,
Max Grolnick, M.D.
Chairman

OBITUARY

JACK S. GOLTMAN

JACK STERNBERG GOLTMAN of Memphis died in his sleep on August 7, 1960. He was 58 years of age and had practiced most of his life in Memphis with the exception of a few years when he served in the Medical Corps of the United States Army during World War II. During the last 15 years of his life he had experienced much sickness and disability and toward the end was spending only a few hours daily in his office.

Dr. Goltman was born in Memphis on October 13, 1901, to the late Dr. and Mrs. Max Goltman. He attended the old Memphis University School. He received his Bachelor of Arts degree and his Master of Arts degree from the University of Pennsylvania. He received his medical degree from Cornell University. His two brothers also became Doctors of Medicine, Dr. Alfred Goltman, who died about 15 years ago, and Dr. David Goltman who is in active practice in Memphis.

During World War II, Dr. Goltman became a Lieutenant Colonel in the Medical Corps of the United States Army. At one time he was Chief of the 12th General Hospital located in the Philippines.

Dr. Goltman was an internist, specializing in allergic diseases. He was active in local and national organizations. He was a member of the Memphis and Shelby County Medical Society, The Tennessee State Medical Association, and the American Medical Association. He was a Fellow of the American College of Physicians and certified by the Board of Internal Medicine. He was a member of the Mid-South and Southwestern Allergy Forums, and also was a Fellow of the American College of Allergists and the International College of Allergists. In addition to his practice, Dr. Goltman also was an instructor at the University of Tennessee. He was a member of St. John's Methodist Church.

Dr. Goltman leaves his wife, Mrs. Ruth Ramply Goltman, two sisters, Mrs. J. D. Shroder of Memphis and Mrs. Maurice Caheen of Birmingham, Alabama, and Dr. David Goltman of Memphis.

The Memphis and Shelby County Medical Society wishes to extend its sincere sympathy to Mrs. Jack Goltman and to other members of the family and to express our regret of this loss to our Society.

ANNOUNCEMENTS

Back Numbers of the Journal of Allergy for the University of Hue Medical School

The University of Hue was founded in 1957 at Hue, the old Imperial Capitol of Vietnam, a few miles south of the seventeenth parallel which divides the free Republic of Vietnam from its Communist counterpart to the north. In the few short years since its opening, the University has expanded from 10 full-time and 30 part-time faculty members and 785 students to 110 professors with 1,192 students enrolled for the 1960-1961 academic year. The University comprises Faculties of Science, Letters and Pedagogy, an Institute of Sinology, and a School of Fine Arts.

With the opening of the 1960-1961 year, the University began its program in medical education. The new School of Medicine is an administrative function of the University and its students have access to the facilities of the 1,100 bed Central Hospital at Hue. The general program will consist of one year of pre-medical study followed by 6 years of medical training, with an emphasis on public health education.

The University has asked the American Friends of Vietnam to attempt to obtain a large quantity of pharmaceuticals and other medical supplies, reference books, professional journals, and other resource materials for its library. Backnumbers of the JOURNAL OF ALLERGY are needed and would be much appreciated. Any such gifts offered to the University will be shipped directly there by the American Friends of Vietnam. The value of all such gifts is deductible from taxable income to the extent and in the manner provided by law.

Please send such contributions to American Friends of Vietnam, 4 West 40th St., New York 18, N. Y.

Buffalo Allergy Society

The Buffalo Allergy Society announces that its new officers for 1961-62 are:

President: Leonard Wolin, M.D., 1201 Colvin Ave., Buffalo 23, N. Y.
Secretary: Robert Ehrenreich, M.D., 1060 Amherst St., Buffalo 16, N. Y.
Treasurer: George Rosenfeld, M.D., 442 Beach Rd., Cheektowaga 25, N. Y.

Cleveland Allergy Society

The newly elected officers of the Cleveland Allergy Society for the year 1961-62 are as follows:

President: Frank S. Houser, M.D.
Secretary-Treasurer: Arthur J. Horesh, M.D.

Michigan Allergy Society

The Michigan Allergy Society at its annual and final meeting of the year elected the following officers for the incoming year 1961-1962:

President: Alex S. Friedlaender, M.D., Detroit
President-Elect: Hilda M. Hensel, M.D., Monroe
Secretary: Israel Wiener, M.D., Detroit
Treasurer: James McLean, M.D., Ann Arbor

The Executive Committee consists of the above officers and the following members: Dr. Robert G. Lovell, Ann Arbor; Dr. Joseph H. Shaffer, Detroit; Dr. Marshall A. MacDonald, Kalamazoo; Dr. John M. Lesesne, Grosse Pointe; and Dr. John B. Holst, Jackson.

California Medical Association: California Society of Allergy

The Joint Annual Meeting of the Allergy Section of the California Medical Association and the California Society of Allergy was held on April 30, 1961, in Los Angeles, Calif.

The following officers were elected for 1961-1962:

President: Gardner S. Stout, M.D., San Mateo
Secretary-Treasurer: Jerome J. Sievers, M.D., Sherman Oaks
Assistant Secretary: Walter R. MacLaren, M.D., Pasadena

The address of the secretary is 4835 Van Nuys, Sherman Oaks, Calif.

Chicago Society of Allergy

At the last meeting of the Chicago Society of Allergy, the following officers were elected:

President: Charles M. Jenkins, M.D.
President-Elect: Abe Matheson, M.D.
Secretary-Treasurer: Ethel M. Davis, M.D.

The address of the secretary is 185 N. Wabash Ave., Chicago 1, Ill.

BOOKS RECEIVED

The Modern Treatment of Asthma. With Special Reference to Gold Therapy. By L. Banszky, M.D., L.M.S.S.A. Bristol, John Wright & Sons Ltd. Ed. 2, Baltimore, 1959, Williams & Wilkins Company. 175 pages. Price, $5.00.

Pulmonary Circulation. An International Symposium sponsored by the Chicago Heart Association, 1958. Edited by Wright R. Adams, M.D., and Ilza Veith, Ph.D. New York and London, 1959, Grune & Stratton. 316 pages. Price, $4.50.

Hypnosis in Skin and Allergic Diseases. By Michael J. Scott, B.S., M.D. Springfield, Ill., 1960, Charles C Thomas, Publisher. 161 pages. Price, $6.50.

Occupational Diseases and Industrial Medicine. By Rutherford T. Johnstone, M.D., and Seward E. Miller, M.D., Philadelphia and London, 1960, W. B. Saunders Company. 482 pages. Price, $12.00.

A System of Medical Hypnosis. By Ainslie Meares, M.D. Philadelphia and London, 1960, W. B. Saunders Company. 484 pages. Price, $10.00.

Essential Hypertension. An International Symposium sponsored by Ciba. Edited by K. D. Bock (Basle), P. T. Cottier (Berne). Berlin, Göttingen, Heidelberg, 1960, Springer-Verlag. 392 pages. Price approximately, $8.00.

Ciba Foundation Study Group No. 6—Metabolic Effects of Adrenal Hormones. Edited by G. E. W. Wolstenholme, O.B.E., M.A., M.B., M.R.C.P., Maeve and O'Connor, B.A. Boston, 1960, Little, Brown & Company. 109 pages. Price, $2.50.

Ciba Foundation Symposium—Adrenergic Mechanisms. Edited by G. E. W. Wolstenholme, O.B.E., M.A., M.B., M.R.C.P., and Maeve O'Connor, B.A. Boston, 1960, Little, Brown & Company. 632 pages. Price, $12.50.

Ciba Foundation Symposium—Haemopoiesis. Cell Production and Its Regulation. Edited by G. E. W. Wolstenholme, O.B.E., M.A., M.B., M.R.C.P., and Maeve O'Connor, B.A. Boston, 1960, Little, Brown & Company. 490 pages. Price, $11.00.

Ciba Foundation Colloquia on Endocrinology. Vol. XIII.—Human Pituitary Hormones. Edited by G. E. W. Wolstenholme, O.B.E., M.A., M.B., B.Ch., and Cecilia M. O'Connor, B.Sc. Boston, 1960, Little, Brown & Company. 336 pages. Price, $9.50.

Inflammation and Diseases of Connective Tissue. A Hahnemann Symposium. Edited by Lewis C. Mills, M.D., and John H. Moyer, M.D. Philadelphia and London, 1961, W. B. Saunders Company. 900 pages. Price, $16.00.

A Manual of Cutaneous Medicine. By Donald M. Pillsbury, M.A., D.Sc.(Hon.), M.D., F.A.C.P., Walter B. Shelley, M.D., Ph.D., F.A.C.P., and Albert M. Kligman, M.D., Ph.D. Philadelphia and London, 1961, W. B. Saunders Company. 430 pages. Price, $9.50.

Medical Almanac 1961/1962. By Peter S. Nagan, A.B., M.A., M.S. Philadelphia and London, 1961, W. B. Saunders Company. 528 pages. Price, $5.00.

How to Master Your Allergy. By Harry Swartz, M.D. Edinburgh, New York, Toronto, 1961, Thomas Nelson & Sons. 288 pages. Price, $5.00.

Current Therapy—1961. Edited by Howard F. Conn, M.D. Philadelphia and London, 1961, W. B. Saunders Company. 806 pages. Price, $12.50.

Allergie in Psychosomatischer und Soziologischer Sicht. Dr. Med. Balthasar Staehelin. Stuttgart, 1961, Georg Thieme Verlag. 120 pages. Price, $3.25.